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MANTLE CELL LYMPHOMA

Clinicopathological Features and Prognostic Factors

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Academic dissertation

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ABBREVIATIONS

ASCT	autologous stem cell transplantation
ATM	ataxia telangiectasia mutated gene
BCL1	B-cell leukaemia/lymphoma 1 (alias: *CCND1, PRAD1*) gene
BCL2	B-cell leukaemia/lymphoma 2 gene
BCL6	B-cell CLL/lymphoma 6 (alias*: LAZ3*) gene
BCNU	carmustine
BEAC	carmustine (BCNU), etoposide, cytarabine (araC), cyclophosphamide
BEAM	carmustine (BCNU), etoposide, cytarabine (araC), melphalan
BMI-1	B-cell-specific Moloney murine leukaemia virus integration site 1 gene
CCND1	coding for cyclin D1 (alias: *BCL1, PRAD1*) gene
CD	cluster of differentiation
CDK	cyclin dependent kinase
CGH	comparative genomic hybridisation
CHOP	cyclophosphamide, doxorubicin (hydroxydaunorubicin), vincristine (Oncovin®), prednisone
CI	confidence interval
CLL	chronic lymphocytic leukaemia
cM	centiMorgan
CMYC	Avian myelocytomatosis viral oncogene
CNOP	cyclophosphamide, mitoxantrone (Novantrone®), vincristine (Oncovin®), prednisone
CNS	central nervous system
COP	cyclophosphamide, vincristine (Oncovin®), prednisone
CR	complete remission
CRP	C-reactive protein
CVP	cyclophosphamide, vincristine, prednisone
DNA	deoxyribonucleic acid
dUTP	deoxyuridine triphosphate
E2F	a transcription factor
EFS	event-free survival
ESHAP	etoposide, methylprednisolone (S-Hydril®), cytarabine (araC), cisplatin (Platinol®)
FADD	Fas-associated via death domain gene
FISH	fluorescence in situ hybridisation
G-CSF	granulocyte colony-stimulating factor
Hpf	high power field
Hyper-CVAD	cyclophosphamide, vincristine, doxorubicin (Adriamycin®), dexamethasone, high-dose methotrexate, high-dose cytarabine
Ig	immunoglobulin
IPI	International Prognostic Index
Kb	kilobase
LAZ3	a zinc finger encoding gene (alias: *BCL6*)
LDH	lactate dehydrogenase
LOH	loss of heterozygosity
MALT	mucosa-associated lymphoma type
M-BACOD	bleomycin, doxorubicin (Adriamycin®), cyclophosphamide, vincristine (Oncovin®), dexamethasone, high-dose methotrexate
MCL	mantle cell lymphoma
mRNA	messenger ribonucleic acid

MTC	major translocation cluster
MZ	mantle zone
ND	not definable
NHL	non-Hodgkin's lymphoma
NR	not reported
NS	not significant
OS	overall survival
p	chromosome short arm
PBSC	peripheral blood stem cell
PCR	polymerase chain reaction
PDCD1	programmed cell death 1 gene
PFS	progression-free survival
PPP2R1B	gene for the β isoform of the A subunit of the serine/threonine protein phosphatase 2A
PR	partial response
PRAD1	parathyroid adenomatosis 1 (alias: *BCL1*, *CCND1*) gene
pRb	retinoblastoma protein
PS	performance status
q	chromosome long arm
RAIDD	RIPK1 domain containing adapter with death domain gene
REAL	Revised European-American Lymphoma Classification
RNA	ribonucleic acid
RR	relative risk
s.c.	subcutaneously
SCT	stem cell transplantation
SLL	small lymphocytic lymphoma
t	translocation
T-PLL	T-cell prolymphocytic leukaemia
TBI	total body irradiation
TTF	time to treatment failure
WF	Working formulation
WHO	World Health Organization
YAC	yeast artificial chromosome

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following studies which are referred to in the text by their Roman numerals. In addition, some unpublished data are presented.

I. Oinonen R., Franssila K., Teerenhovi L., Lappalainen K., Elonen E. Mantle cell lymphoma: clinical features, treatment and prognosis of 94 patients. *Eur J Cancer* 34:329-336, 1998.

II. Oinonen R., Franssila K., Elonen E. Central nervous system involvement in patients with mantle cell lymphoma. *Ann Hematol* 78:145-149, 1999.

III. *Monni O., *Oinonen R., Elonen E., Franssila K., Teerenhovi L., Joensuu H., Knuutila S. Gain of 3q and deletion of 11q22 are frequent aberrations in mantle cell lymphoma. *Genes Chromosomes Cancer* 21:298-307, 1998.†

IV. Räty R., Franssila K., Joensuu H., Teerenhovi L., Elonen E. Ki-67 expression level, histological subtype, and the International Prognostic Index as outcome predictors in mantle cell lymphoma. *Eur J Haematol (in press).*

V. Räty R., Franssila K., Jansson S-E., Joensuu H., Wartiovaara-Kautto U., Elonen E. Predictive factors for blastoid transformation in the common variant of mantle cell lymphoma. *Submitted.*

VI. Oinonen R., Jantunen E., Itälä M., Lehtinen T., Kuittinen O., Franssila K., Wiklund T., Elonen E. Autologous stem cell transplantation in patients with mantle cell lymphoma. *Leuk Lymphoma* 43:1229-1237, 2002.

* These authors contributed equally to the study.

† This study is included also in the thesis of Outi Monni entitled *Changes is DNA sequence copy number in diffuse large B-cell and mantle cell lymphoma (Helsinki 1998)*

ABSTRACT

Mantle cell lymphoma (MCL), characterised by the t(11;14)(q13;q32), was established as a subtype of non-Hodgkin's lymphoma in the early 1990's. There is still much to be learned about this novel disease entity, in particular, there is a lack of knowledge about the prognostic factors and optimal treatment of MCL. The aim of this thesis was to investigate the clinicopathological features, treatment results, and outcome of MCL patients, and to examine the relative prognostic value of several clinicopathological factors.

This retrospective series consists of 127 consecutive patients diagnosed with MCL in the Helsinki University Central Hospital in 1980 - 1999. The median age of the patients was 65 years, and 83% presented with advanced disease. Of the actively treated patients 45% achieved complete remission, but the median duration of remission was only 16 months. The long-term prognosis was poor with a median survival of 34 months. First-line treatment with anthracycline-containing therapy showed no clear survival benefit over chemotherapy not containing anthracycline. Three histological subtypes of MCL were defined and these types were associated with prognostic significance: 1) the mantle zone/nodular subtype of the common variant was diagnosed in 19% of the patients and the median survival time was 70 months. 2) The diffuse subtype of the common variant (64%) was associated with a median survival of 35 months, and 3) the blastoid variant (17%) with a median survival of 11 months ($p < 0.001$). A high cell proliferation index was associated with the blastoid variant and poor survival in general. A subset of common MCLs had also enhanced proliferation as assessed by a high Ki-67 expression (≥ 26% of cells, the upper tertile), which predicted short survival (median 20 vs. 45 months, $p < 0.001$) in these patients. The results of multivariate analyses suggested that the International Prognostic Index may not be an optimal prognostic tool for MCL, and that a better prognostic index might be obtained by combining Ki-67, and possibly the histological subtype, together with age, stage, and serum LDH (lactate dehydrogenase).

Blastoid transformation during the course of the disease occurred in 35% (18/52) of the patients. The minimum estimated risk of transformation was 42% at 5 years. An elevated serum LDH level, leukocytosis, and a high cell proliferation rate at diagnosis were associated with an increased risk of transformation. Importantly, CNS involvement was associated with the blastoid morphology in five of the seven patients who developed CNS relapse during the follow-up.

DNA copy number changes were studied by comparative genomic hybridisation (n= 34) and these changes turned out to be complex but concentrated to highly specific regions. A gain in 3q (52%) and a deletion at 11q22 (30%) were the most frequent and important

findings. The mean number of changes was higher in the blastoid variant than in the common MCL, as well as in samples taken at relapse than in those taken from untreated lymphoma of the same patient.

Of the 48 consecutive MCL patients originally scheduled for autologous stem cell transplantation (ASCT) in five Finnish transplantation centres in 1995-2000, altogether 35 patients underwent ASCT. ASCT was an effective treatment: the overall response rate was 94%, and the expected 4-year survival 69%. However, there was no plateau in the event-free survival curve after ASCT. Patients transplanted after relapse had a similar outcome than those transplanted as first- or second-line treatment for lymphoma. An elevated serum C-reactive protein level at diagnosis and an age over 60 years at transplantation were associated with a poor outcome after ASCT.

INTRODUCTION

Non-Hodgkin's lymphomas (NHLs) are a heterogeneous group of lymphoid malignancies with large variation in course of the disease and prognosis [1]. During past decade increasing knowledge of the biology of the immune system and molecular genetics related to NHLs have led to the recognition of a number of new clinicopathological entities [2, 3]. One of these is the mantle cell lymphoma (MCL), which was recognised in the beginning of 1990's as a distinct subtype of B-cell lymphoma characterised by a specific translocation t(11;14)(q13;q32) [4, 5]. MCL has been estimated to represent 7 - 9% of all NHLs in Europe and a somewhat lower proportion, about 2 - 6%, in the USA [6].

MCL is typically a disease of middle or old age. It is usually diagnosed at an advanced stage. The outcome is poorer than in any of the other small cell lymphoma [7, 8]. Although clearly a distinct clinicopathological entity, the histopathological features of MCL vary. The growth pattern of the lymphoma is typically diffuse but may be also nodular, or the lymphoid cells may proliferate as wide collars surrounding the reactive germinal centres; this type of growth is called the mantle zone pattern. In addition to the common (or typical) MCL, a cytomorphologically more aggressive blastoid variant is recognised [2, 3], which is associated with a highly aggressive clinical course [7, 9-13]. Nevertheless, the association between different growth patterns and patient outcome is still poorly understood. It also appears that histological progression from the common to the blastoid variant is not uncommon [9, 10, 14].

The MCL-related t(11;14) leads to the overexpression of the cell-cycle regulatory protein cyclin D1, but this alone does not seem to explain the poor prognosis of patients with MCL. Abnormalities of p53 and inactivation of some cyclin-dependent kinase inhibitors have found to be associated with the aggressive variants of MCL and a poor prognosis [15-18], but the cytogenetic and molecular changes in MCL are still poorly understood. An imbalance between cell proliferation rate and cellular apoptosis may be involved, and disturbances of several apoptotic pathways have indeed been suggested to contribute to the pathogenesis of MCL [19].

As in other NHLs, there are some clinical factors that have been reported to have predictive value in MCL, such as age over 60, advanced disease stage, poor performance status (PS), and elevated serum lactate dehydrogenase level (LDH) at the time of the diagnosis [9, 11, 14, 20, 21]. However, identification of universally valid prognostic factors has been difficult, because most of the series thus far have included a relatively small number of patients. In particular, the effect of histopathological features on patient outcome in conjunction with clinical factors has not been established.

There are no generally accepted optimal treatment strategies for MCL. It is still unclear whether anthracycline-containing regimens improve the survival of patients with MCL. However, none of the conventional treatments has been found to be curative, and more effective treatments are needed [6, 22, 23]. In younger patients high-dose therapy followed by autologous or allogeneic stem cell transplantation (SCT) has been considered to be a means to improve the poor prognosis, but the role of intensive therapy in the treatment of MCL is still unsettled. There is only limited information available on the ideal timing of SCT and on the factors that predict outcome after transplantation.

The aim of this thesis is to improve our understanding of the clinical, histopathological, and genetic features of MCL, and to investigate their prognostic significance for patients with MCL.

REVIEW OF THE LITERATURE

EVOLVING CLASSIFICATION OF NON-HODGKIN'S LYMPHOMAS

The non-Hodgkin's lymphomas (NHLs) are a diverse group of diseases caused by malignant proliferation of lymphatic cells. In about 85% of the cases the disease affects the B-lymphocyte lineage and in about 15% the T-lymphocyte lineage. Lymphomas may originate from primitive stem cells (precursor B- or T-cell neoplasms) or from cells at later stages of maturation after stem cell differentiation (mature B- or T-cell neoplasms). An increasing understanding of the biological features of normal lymphocytes progressing through differentiation pathways and their genetic abnormalities related to the malignant transformation have permitted a more precise definition of the subtypes of NHLs [2, 3].

The widely used classification of lymphomas by Rappaport based on the morphological features of the lymphomas was published in 1960s [24]. Since then several classifications have followed. The complexity of the lymphoid system has lead to various systems to categorise lymphomas, and several different classifications have been used simultaneously by the medical profession. Among the most widely used classifications have been the Kiel classification from 1974 (revised in 1988) [25] and the Working Formulation for Clinical Usage (WF) from 1982 [26]. In WF, the lymphomas were categorised according to the estimated clinical aggressiveness of the disease into three grades: low-grade, intermediate-grade, and high-grade malignant lymphomas. In the Kiel classification, on the other hand, the lymphomas were grouped according to their histopathological features into either a low grade or high grade of malignancy. The WF classification became the most widely used classification in the North America, whereas the Kiel classification dominated clinical practice in Europe [1].

The uncertainties of classification have resulted in heterogeneous categorisation of many lymphoma entities, variable nomenclature and variable diagnostic criteria. The advances in immunology and molecular genetics of lymphoid neoplasms over the last decades have allowed characterisation of new and previously unrecognised types of NHL. The Revised European-American Lymphoma (REAL) classification proposed by the International Lymphoma Study Group was published in 1994 to provide a unified international basis for both clinicians and investigators to categorise lymphomas [2]. In this classification lymphomas are defined for the first time by a combination of morphologic, immunophenotypic, genetic, and clinical attributes. One important result from this new approach was the recognition that lymphoma entities may have a range of morphological grades and express varying clinical aggressiveness. The classification principles of the International Lymphoma Study Group are used in the recently

published World Health Organization (WHO) classification (Table 1), which represents the first true global consensus on the classification of tumours of haematopoietic and lymphoid tissues [3].

Table 1. *The WHO classification of lymphoid neoplasms [3].*

B-CELL NEOPLASMS	**T- AND NK-CELL NEOPLASMS**
Precursor B-cell neoplasm • Precursor B-lymphoblastic leukaemia / lymphoma	**Precursor T-cell neoplasms** • Precursor T-lymphoblastic leukaemia / lymphoma • Blastoid NK-cell lymphoma
Mature B-cell neoplasms • Chronic lymphocytic leukaemia / small lymphocytic lymphoma • B-cell prolymphocytic leukaemia • Lymphoplasmacytic lymphoma • Splenic marginal zone lymphoma • Hairy cell leukaemia • Plasma cell myeloma / Plasmacytoma • Extranodal marginal zone B-cell lymphoma (MALT lymphoma) • Nodal marginal zone B-cell lymphoma • Follicular lymphoma • Mantle cell lymphoma • Diffuse large B-cell lymphomas • Mediastinal (thymic) large B-cell lymphoma • Intravascular large B-cell lymphoma • Primary effusion lymphoma • Burkitt lymphoma/leukaemia	**Mature T-cell neoplasms** • T-cell prolymphocytic leukaemia • T-cell large granular lymphocytic leukaemia • Aggressive NK-cell leukaemia • Adult T-cell lymphoma/leukaemia (HTLV1+) • Extranodal NK- / T-cell lymphoma, nasal type • Hepatosplenic T-cell lymphoma • Subcutaneous panniculitis-like T-cell lymphoma • Mycosis fungoides / Sézary syndrome • Primary cutaneous anaplastic large cell lymphoma • Peripheral T-cell lymphoma, not otherwise specified • Primary systemic anaplastic large cell lymphoma
B-cell proliferations of uncertain malignant potential • Lymphomatoid granulomatosis • Post-transplant lymphoproliferative disorder, polymorphic	**T-cell proliferation of uncertain malignant potential** • Lymphomatoid papulosis
HODGKIN LYMPHOMA • **Nodular lymphocyte predominant Hodgkin lymphoma** • **Classical Hodgkin lymphoma** Nodular sclerosis Hodgkin lymphoma Lymphocyte-rich classical Hodgkin lymphoma Mixed cellularity Hodgkin lymphoma Lymphocyte depleted Hodgkin lymphoma	

MANTLE CELL LYMPHOMA

Background

In the beginning of the 1990s the NHL subtypes termed as intermediate lymphocytic lymphoma, mantle zone lymphoma, or centrocytic lymphoma (based on morphological, immunohistological, and cytogenetic features) were considered to comprise a single distinct subtype of B-cell NHL [4, 5]. To unify these terms under one entity the name mantle cell lymphoma (MCL) was proposed, and MCL was incorporated for first time as a unique clinicopathological entity in the REAL classification in 1994 [2]. Immunological studies had suggested that neoplastic cells in MCL do not originate from the centrocytes, as thought previously, but rather that the normal cell counterpart of MCL is a naive pre-germinal centre cell in the primary lymphoid follicles or the mantle zones of secondary follicles [4, 5, 27]. Thus, the name mantle cell lymphoma was taken into use. However, recent studies have detected somatic mutations of the immunoglobulin variable region genes in some tumours of patients with MCL suggesting a follicular / post-follicular origin in these MCLs [28-31].

According to the Kiel classification [25], MCL (identified as diffuse centrocytic lymphoma) belongs to the group of low-grade lymphomas but, in spite of its nonaggressive histological features, it carries a poorer prognosis than the other small cell lymphomas. In the WF classification [26] MCL is not recognised as a distinct disease entity, but it has been included in the subgroup of diffuse small cleaved cell or diffuse mixed small and large cell lymphomas of intermediate grade of malignancy, or in the group of follicular low-grade lymphomas. In the REAL / WHO classification, MCL is recognised as having a range of morphological grades; although MCL is not formally graded for clinical purposes, one morphologically more aggressive variant, the blastoid variant of MCL is considered to be of clinical significance [3].

Pathogenesis and biology

Translocation (11;14)(q13;q32) and *BCL1/CCND1* gene

The characteristic cytogenetic alteration in MCL is the t(11;14)(q13;q32) [32, 33]. Classical cytogenetic studies detect t(11;14) in up to 65% of MCLs [34, 35], but recent studies using a variety of FISH techniques have shown that this translocation is present in nearly all MCLs [36-38]. Although t(11;14) is extremely rare in other lymphomas, it is not totally specific for MCL, since it has been detected in occasional atypical cases of chronic lymphocytic leukaemia (CLL) associated with an aggressive clinical course, as well as in up to 20-30% of the prolymphocytic leukaemias and in 5% of multiple myelomas [35, 39].

In the t(11;14) the immunoglobulin heavy-chain joining region in chromosome 14 is juxtaposed adjacent to a region on 11q13 designated *BCL1* (B-cell lymphoma / leukaemia 1) [40-43]. The breakpoints in the *BCL1* locus occur in 30 - 55% of the cases in the major translocation cluster (MTC) region, but additional breakpoints distal to this region are detected in 10 - 20% of the cases [44-46]. A new gene named *PRAD1* located approximately 120kb downstream of the MTC breakpoint was first identified in studies on parathyroid adenomas with inversion in chromosome 11, and this gene was considered to be a putative oncogene deregulated by the t(11;14) [47-49]. In further studies, the *PRAD1* sequence was recognised as having a high degree of homology with cyclins, and the new member in that gene family was renamed *CCND1* encoding for the cyclin D1 protein [48, 50]. In t(11;14) the coding region of *CCND1* is structurally intact, but the chromosomal rearrangement positioning the *CCND1* gene adjacent to the enhancer region of the immunoglobulin heavy-chain gene results in upregulation of *CCND1* and in increased expression of the cyclin D1 protein [33, 51].

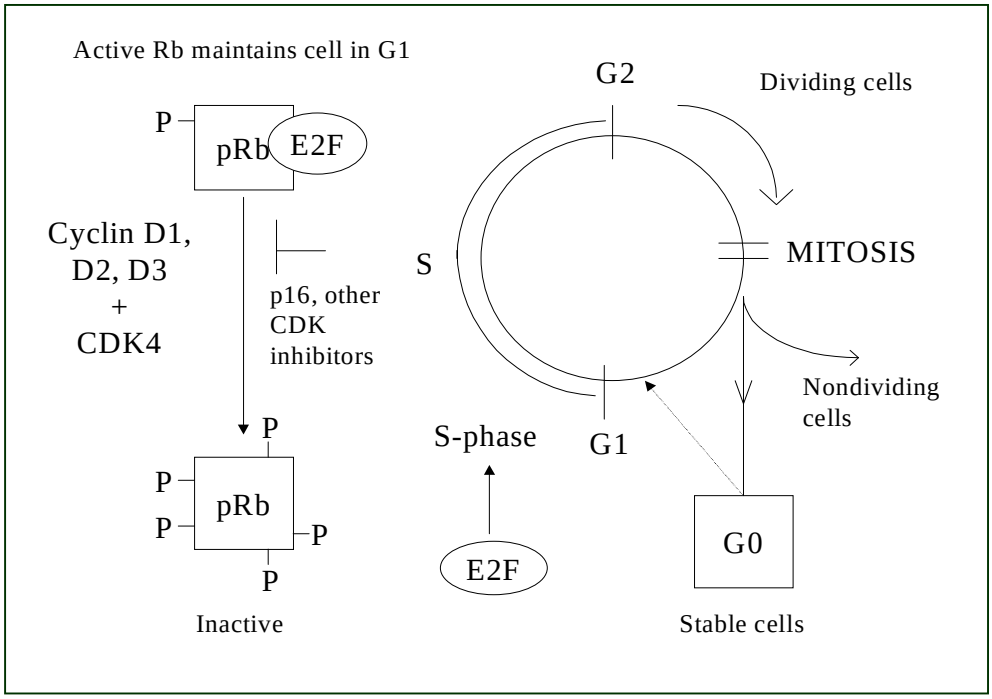
Cyclin D1 protein and aberrant cell cycle function

In normal lymphoid cells the RNA and protein levels of cyclin D1 are extremely low or absent [52-54]. Although the oncogenic mechanism of cyclin D1 is not completely understood, the constant expression of cyclin D1 has an important role in the pathogenesis of MCL since cyclin D1 promotes the progression of cells through the main commitment checkpoint in G1- to S- phase of the cell cycle [32, 43, 55].

Regulation of the cell cycle. Cell replication is controlled by complex feedback pathways that regulate the passage of cells through the four phases of the cell cycle called the G1-, S-, G2-, and the M-phase (G stands for gap, S for synthesis and M for mitosis). Various combinations of complexes of cyclins and cyclin dependent kinases (CDKs) are essential for the progression of the cell cycle through a number of various cell cycle checkpoints. The cyclin/CDK -complexes are negatively regulated by cyclin dependent kinase inhibitors, such as p15, p16, p18, and p21. There are at least eight families of mammalian cyclins (cyclins A-H) acting during the various stages of the cell cycle. Of these, cyclins D1-D3 belong together with cyclins C and E to the so called G1-cyclin family that function mainly in late G1-phase [1].

Cyclin D1 and the cycling cells. Cyclin D1 binds to CDK4 or CDK6 forming a cyclin/CDK complex, which binds to the retinoblastoma protein (pRb). This leads to phosphorylation of pRb and inactivation of its suppressor effect on cell-cycle progression. Transcription factor E2F is normally bound to the unphosphorylated pRb, but as a result of hyperphosphorylation of pRb, E2F is released and drives the cell to the S-phase (Figure 1) [33, 55]. Concordantly, although pRb seems to be normally expressed [56], E2F overexpression has been detected in a large proportion of MCLs [18].

Figure 1. *Schematic representation of the phases of the cell cycle and the functional relationship of cyclin D1 in regulation of the cell cycle.*



Other cytogenetic and molecular changes

Although the t(11;14) leads to overexpression of cyclin D1 protein and although this is clearly important in the oncogenesis of MCL, this alone does not lead to development of B-cell lymphoma in transgenic mice; cooperation with another oncogene, *CMYC*, is required [57, 58]. However, although *CMYC* mRNA overexpression has been found in a subset of MCLs, no structural gene alterations of the *CMYC* seem to be involved in the pathogenesis of MCL [59]. Cells overexpressing cyclin D1 have abnormal proliferative characteristics and a shortened G1-phase, but usually the cell proliferation rate in MCLs is relatively low [5, 22]. Moreover, there is no correlation between cyclin D1 expression level and cell proliferation rate [32, 56, 60], suggesting that other cellular factors in addition to aberrant expression of cyclin D1 are involved. Recently, an increased effect on cell proliferation was described when MCL cells, expressing CD40 like most B cells, were cultured with soluble recombinant CD40 ligand [61], and this growth-effect was found to be enchanced when the cells were further exposed to the cytokines interleukin-4 or interleukin-10 [62, 63].

p53 and CDK inhibitors. The *p53* gene acts as a tumour suppressor by modulating apoptosis. In case of DNA damage, *p53* activates transcription of the $p21^{Cip1}$ (also named $p21^{Waf1}$ or $p21^{Sdi1}$) leading to accumulation of p21 protein, which is an important inhibitor of the cyclin/CDK complexes. Similarly, if the cell fails to repair the DNA

damage during the pause in cell replication induced by p21 accumulation, *p53* may trigger cell suicide through apoptosis [64-66].

Abnormalities of *p53* are found in more than 50% of all human tumours [1]. Some studies have shown *p53* mutations and p53 protein overexpression to occur also in the aggressive variants of MCL, where the effect of overexpressed cyclin D1 may be enhanced by loss of cyclin-CDK inhibition as a result of the *p53* mutation [15-17, 67]. Similarly, *p21* expression is lost in some aggressive variants of MCL, although the relationship of this aberration to *p53* mutations is not clear [68]. The p27 protein may also be inactivated; this protein belongs together with p21 and p57 to the same CIP/Kip family of CDK inhibitors. In one recent series consisting of 112 patients with MCL, over 80% had a total or partial loss of p27 protein expression [18]. p27 mRNA expression was normal in this study, suggesting that the low levels of p27 resulted from increased degradation of activity via the proteasome pathway.

Alternative pathogenetic mechanisms independent of p53 may also be operative in MCL. Aberrations of another CDK inhibitor, $p16^{INK4a}$, have been detected in aggressive variants of MCL with the normal, wild-type *p53* gene [68, 69]. Similarly, amplification and expression of the putative oncogene *BMI-1*, which acts as an upstream negative regulator of $p16^{INK4a}$ and $p14/p19^{ARF}$ gene expression, was recently reported to occur more often in MCL than in other types of NHLs [70]. However, the role of the *BMI-1* gene in lymphomagenesis has not been established. *BMI-1* gene alterations were identified only rarely in MCL, and there was no relationship between BMI-1 and $p16^{INK4a}$ mRNA levels [70]. Alterations of *p19* and *p18*, other members of the INK4a CDK inhibitor family, have been detected only rarely in MCL [33, 71].

Abnormalities of p53 protein and inactivation of CDK inhibitors, except for p27, have been observed only seldom in MCL and mostly only in the aggressive variants. The significance of these molecular changes for the pathogenesis of MCL is not yet fully understood, but they are believed to be more important for the progression of lymphoma than for primary lymphomagenesis of MCL.

DNA copy number changes. Several studies using comparative genomic hybridisation (CGH) and/or fluorescence in situ hybridisation (FISH) to characterise alterations in the frequency of DNA copy number sequences in MCL have been published [study III, 72-75]. Although the results have differed somewhat, these studies have confirmed a characteristic profile of chromosomal changes in MCL different from that in other lymphomas. For example, in addition to trisomy 12, deletions in 13q, 11q, 6q, and 17 p are the most frequent aberrations in CLL [76], whereas studied by CGH gains at chromosomes X, 1q, 7, and 3, together with losses in 6q, X, and 1p have been reported in diffuse large B-cell lymphoma [77]. In contrast, in MCL the most frequent chromosomal imbalances are gains at chromosomes 3q (49-70%), 8q (22-30%), and 12q (20-30%), and the most frequent losses of chromosomes 1p (24-33%), 6q (27-37%), 9p

(16-41%), 11q (22-31%), and 13q (41-69%) [study III, 72-75]. DNA amplifications and the total number of copy number changes have been reported to be higher in the blastoid variant than in the common variant of MCL [72]. In line with findings on the gene level, loss of 17p has been found to correlate with the rate of *p53* inactivation, gain of 12q with the frequency of *CDK4* amplification, and high-level amplification of 10p12-13 with *BMI-1* amplification [72]. Interestingly, further studies on the deletion 11q (by FISH using a contig of YAC probes) showed deletion of 11q22-23 in as many as 49% of MCL samples [78]. This region is known to harbour a number of tumour-associated genes, including the ataxia telangiectasia mutated (*ATM*) gene (discussed below). These commonly affected chromosomal regions may also harbour unknown oncogenes or tumour suppressor genes.

Deletion of 11q22-23 and the *ATM* gene. The *ATM* (ataxia telangiectasia mutated) gene is located at 11q22-23, and encodes for a large kinase that is important for DNA damage recognition and is required for checkpoint control of the cell cycle to reduce the progression of cells harbouring damaged DNA [79]. Mutations and deletions of the *ATM* gene have been found in a variety of sporadic neoplasms, including 46 - 67% of T-PLL and 19 - 34% of CLL [80-85]. More recently, *ATM* mutations have been identified in all 7 MCLs with a 11q22-23 deletion and in 2 of the 5 MCLs with no 11q deletion [86], and in another study in 6 of 9 MCLs with a loss of 11q, and in 2 of 11 lymphomas with a normal chromosome 11 profile [87]. Consequently, it has been suggested that loss of this tumour suppressor gene may play an important role in the pathogenesis of MCL and some other lymphoid malignancies.

Bcl-2 protein. *BCL2* is a proto-oncogene, the activation of which prevents apoptosis and may lead to abnormal cell survival with accumulation of additional genetic lesions; this may ultimately lead to lymphomagenesis. The t(14;18) is a genetic hallmark of follicular lymphoma. This translocation leads to deregulated expression of *BCL2*, and to high levels of the Bcl-2 protein [88]. However, overexpression of Bcl-2 protein can be seen also in the absence of the t(14;18) in many other (low-grade) B-cell lymphomas including MCL [89].

Other apoptotic pathways. Recently, a report was published on the use of an oligonucleotide microarray to compare the expression of some apoptosis-related genes in five MCLs with the expression in four non-malignant hyperplastic lymph nodes [19]. In addition to the Bcl-2 pathway other apoptotic pathways were found to be altered in MCL. The apoptosis-related genes *FADD* and *PDCD1* were down-regulated and this was suggested to lead to the lack of appropriate regulation of apoptosis and to the accumulation of abnormal cells. The *RAIDD* gene, an inductor of apoptosis in mammalian cells, was also found to be down-regulated in MCL. Overall, these results suggest that, in addition to the enhanced cell cycle progression caused by overexpressed cyclin D1, disturbances in several apoptotic pathways may contribute to the molecular

genetic pathogenesis of MCL and lead to an imbalance between cell apoptosis and cell survival.

Pathological characteristics

Morphological features. The phenotype of MCL covers a large spectrum of morphological appearance: lymphomas with slowly proliferating, small-sized round lymphocytes at one end and large-sized pleomorphic cells and a high mitotic index at the other end [6, 22, 90]. Typically, as defined in the WHO classification, MCL is "a B-cell neoplasm composed of monomorphous small to medium-sized lymphoid cells with irregular nuclei, which morphologically most closely resemble centrocytes/cleaved follicular centre cells but which often have at least slightly less irregular nuclear contours. Neoplastic transformed cells, paraimmunoblasts and pseudofollicles/proliferation centres are absent" [3]. In cases with a more aggressive histological picture, i.e., lymphoid cells with medium to large-sized nuclei and usually with a high mitotic index, the term blastoid or lymphoblastoid variant of MCL has been applied because the cells resemble lymphoblasts. There is also a separate type of blastoid variant called the pleomorphic variant [3, 60]. This variant is characterised by a more heterogeneous proliferation of cells with large cleaved to oval nuclei and a pale cytoplasm. Usually, both the lymphoblastoid and the pleomorphic lymphomas are included in the blastoid variant.

The growth pattern of MCL is typically diffuse or vaguely nodular, but sometimes a more prominent nodular pattern resembling the architecture that is usually seen in follicular lymphoma is present. Rarely the lymphoma cells proliferate as wide collars surrounding the reactive germinal centres throughout the lymph node, giving the name mantle zone pattern [3, 4, 91-94]. Some authors believe that the mantle zone pattern represents through a nodular phase the initial stage of the disease, leading to the diffuse pattern of MCL [6].

Immunophenotype. The neoplastic cells typically express B-cell associated antigens, e.g., CD19 and CD20, and surface immunoglobulins IgM and, usually, IgD. In most cases the cells are positive for the T-cell specific antigen CD5, but they do not express CD10 or CD23. Lambda-clonality is seen slightly more often than kappa-clonality. Bcl-2 is universally positive [93-97]. Almost all cases of MCLs express cyclin D1 protein [32, 52-54, 98, 99].

Histological progression. Histological progression to the typical large cell lymphoma does not occur in MCL, but progression from the common to the blastoid variant of MCL during the course of the disease does occur. In the few studies where serial biopsies have been taken during the course of the disease, blastoid transformation has been reported in 19 to 29% of the patients [9, 10, 14, 100]. Blastoid transformation at any time during the course of the disease has been shown to be associated with a poor

survival [9], but the clinical features, or the predictive factors related to the blastoid transformation per se have not been characterised.

Diagnosis and differential diagnosis of mantle cell lymphoma

MCL may be easily confused with other types of B-cell NHLs because of its morphological heterogeneity and resemblance to other lymphomas. For an accurate diagnosis of MCL the combination of morphology and immunological or molecular studies are needed [2, 3, 5, 101].

It may sometimes be difficult to differentiate between follicular lymphoma and MCL with a more or less nodular pattern. MCL cells are usually not as cleaved as in follicular lymphoma, and large transformed cells are usually absent in MCL. In immunophenotyping, CD5 positivity and the absence of CD10 in MCL are useful, since in follicular lymphoma the reverse is often true. In atypical cases of small lymphocytic lymphoma (SLL)/CLL (without the characteristic pseudofollicular proliferation centres) morphological differentiation from MCL may be extremely difficult. Expression of CD5 is not helpful, since it is usually present in both MCL and SLL/CLL, but SLL/CLL typically expresses the CD23 antigen, whereas it is absent in most cases of MCL [102, 103]. Occasionally MCL may also be confused with marginal zone lymphoma (mucosa-associated, MALT-lymphoma), especially when situated in the gastrointestinal tract. Here again, the typical immunophenotype of MCL is helpful for differential diagnosis. As the name blastoid variant of MCL emphasises, in these lymphomas, the MCL cells show cytologic resemblance to lymphoblasts, and may be misdiagnosed as lymphoblastic lymphoma, unless proper immunophenotyping is carried out.

Cyclin D1 plays an important role in the diagnosis of MCL. The t(11;14) is present in almost all cases of MCL, but currently the methods needed to demonstrate this translocation are not widely available for clinical practice. On the other hand, overexpression of cyclin D1 protein can be easily demonstrated in almost all cases of MCLs, and overexpression of this cyclin is rare in other lymphoproliferative diseases [32, 52, 54, 98, 104, 105]. Immunohistochemical detection of cyclin D1 in paraffin-embedded tissue sections has been found to be only slightly less sensitive than mRNA analysis [53, 106]. Cyclin D1 expression may be demonstrated reliably also from small tumours and bone marrow biopsies if optimal techniques are used [107, 108]. Recently, a flow cytometry method to detect cyclin D1 overexpression using the monoclonal antibody 5D4 has been described [109]. New quantitative PCRs have been introduced as rapid, specific, and sensitive diagnostic methods for the detection of cyclin D1 overexpression [110-112].

Clinical manifestations

Typically MCL presents in middle-aged or elderly patients at an advanced disease stage and generalised lymphadenopathy [9-11, 14, 20, 21, 113-115]. Male predominance varying from 67 to 94% is reported in most studies. Extranodal manifestations of lymphoma are common, and more than half of the patients present with bone marrow involvement. The pattern of bone marrow infiltration is usually intertrabecular with nodular or interstitial infiltrates, but tumour cells may also be distributed paratrabecularly and diffusely, or as a combination of these [10, 116, 117]. Although bone marrow involvement is common, leukaemic disease is rather unusual at the time of the diagnosis [118-120]. Another typical extranodal site of the disease is the gastrointestinal tract, where the lymphoma typically presents as multiple polyps, a condition known as *lymphomatoid polyposis* [121-125]. Expression of the mucosal homing receptor integrin $\alpha 4\beta 7$ may be associated with digestive tract involvement. In a small study of 13 patients, all seven patients with gastrointestinal involvement of MCL showed expression of $\alpha 4\beta 7$ while none of the six patients with no gastrointestinal involvement expressed this integrin [126]. Other predilection sites are the spleen, Waldeyer's ring, and the liver. The spleen may be remarkably enlarged, and a spontaneous rupture of the spleen may occur [9, 127, 128].

Central nervous system (CNS) involvement has been considered to be very rare in small cell lymphomas [129-132]. CNS manifestation at the time of the diagnosis of MCL has been reported only in occasional patients in the literature [95, 133, 134], but the possibility that the incidence of CNS lymphoma in MCL patients might be higher than this has been raised by a Spanish group, who reported five (23%) out of 22 patients and later seven (12%) of 59 MCL patients to develop CNS involvement during the course of the disease [11, 135]. The CNS relapse was a relatively late event in the natural course of the disease: the median time of onset of CNS manifestation was 18 months after the diagnosis of MCL.

Treatment results and prognosis

Conventional treatment

MCL brings together the worst characteristics of high-grade and low-grade lymphomas: the course of the disease is not indolent and the lymphoma is only rarely curable. Although chemosensitivity of MCL is moderate and complete remissions (CR) range from 20 to 50% in different series, the duration of remission is usually short. There is no plateau in the survival curves, the median survival time is 3 - 4 years only, and less than 10% of all patients are alive 10 years after the diagnosis of MCL [7, 9, 11, 21, 113, 136, 137].

There are no universally approved evidence based treatment strategies for MCL. It is still unclear whether anthracycline-containing chemotherapy regimens improve the outcome (Table 2). In some retrospective studies [14, 21] anthracycline-containing treatment has been associated with a better outcome than non-anthracycline regimens, but not in others [9, 11, 20]. In a prospective randomised trial on the treatment of advanced low-grade NHLs, the CR-rate was 26% (5/19) in MCL patients treated with prednimustine and mitoxantrone, as compared to 5% (1/19) in those treated with COP, but the overall response rates were similar [138]. There was no significant difference of outcome between 37 patients treated with COP and 27 patients treated with CHOP in a prospective randomised trial involving patients with advanced centrocytic lymphoma [139]. Other treatment results with the standard CHOP regimen have also been inconclusive. A significantly worse outcome was reported among patients with MCL than any other patients with low- or intermediate-grade lymphoma (reclassified as WF categories A through E) treated with CHOP in a study consisting of 376 patients [7].

Fludarabine. Fludarabine is one of the newer purine analogs which are highly active in the treatment of CLL and low-grade NHLs [140-143]. However, in MCL the efficacy of fludarabine seems to be only moderate. The overall response rates to fludarabine treatment range from 33 to 63% when used as a single agent [144-146]. There was no difference in outcome when the combination of fludarabine and idarubicine was compared to single-agent fludarabine as frontline treatment of patients with MCL [147]. Instead, encouraging preliminary results were recently reported for a combination consisting of cisplatine, fludarabine, and cytarabine in a small phase II study on refractory NHLs which included eight MCL patients [148].

Hyper-CVAD. Considerably better results than those obtained with CHOP therapy have recently been reported with the Hyper-CVAD regimen (including hyperfractionated intense-dose cyclophosphamide, doxorubicin, vincristine, and dexamethasone, alternating with high doses of cytarabine and methotrexate), which was originally designed to treat patients with acute lymphoblastic leukaemia (Table 2). In a series of 45 patients an overall remission rate of no less than 94% was seen after four cycles, and in 25 previously untreated patients under 66 years of age following stem cell transplantation, the three-year overall survival (OS) was 92% and the event-free survival (EFS) 72% three years after treatment. The corresponding rates in a historical control group of previously untreated patients who had received CHOP or CHOP-like treatment were 56% and 28% [149]. Encouraging results with Hyper-CVAD have also been published in patients over 65 years of age who did not undergo stem cell transplantation [150], but the superiority of these regimens over CHOP needs to be verified in randomised studies, because case selection may introduce bias, and comparisons to historical controls are notoriously difficult.

Table 2. *Results of chemotherapy in MCL.*

Study	N of treated patients	CR-rate	Median EFS/PFS (months)	Median OS (months)
Prospective randomised trials				
Meusers *et al.* (1989) [139]				
CHOP	27	58%	7	37
COP	37	41%, p = NS	10 p = NS	32 p = NS
Unterhalt *et al.* (1996) [138]				
PmM	19	26%[a]	] 8	] 28
COP	19	5%[a]		
Reviews of randomised trials				
Teodorovic *et al.* (EORTC) (1995) [137]				
CHVmP-VB / ProMACE-MOPP[b]	29	52%	19	45
CVP / CVP + interferon α	35	40% p = NS	20 p = NS[b]	45 p = NS[b]
Comparison with historical controls			Survival fractions	
Khouri *et al.* (1998) [149]			at 3-years	at 3-years
Hyper-CVAD[c]	25	100%	72%	92%
CHOP (historical control)	25	NR	28% p = 0.0001	56% p = 0.05
Reviews of one arm therapy studies			Survival fractions	
Fisher *et al.* (SWOG) (1995) [7]			at 10-years	at 10-years
CHOP	36	NR	6% (FFS)	8%
Vandenberghe *et al.* (1997) [151]				
Chlorambucil, COP, RT	65	45%	24	57
Retrospective series				
Berger *et al.* (1994) [20]	52	31%	14	52
Anthracyclines vs. fludarabine vs. others	19 / 11 / 22	NR	No difference	No difference
Norton *et al.* (1995) [9]	66	9%	10	36
Anthracyclines vs. others	12 / 54	25 vs. 6% p = NS	No difference	No difference
Zucca *et al.* (1995) [21]	65	51%	44 (DFS)	42
Anthracyclines vs. others	33 / 28	68 vs. 25% p = 0.001	Favour for A+, p = 0.009	~ 52 vs. ~30 p = 0.003
Bosch *et al.* (1998) [11]	59	19%	NR	49
Anthracyclines vs. others	38 / 16	15 vs. 21% p = NS	-	46 vs. 69 p = NS
Samaha *et al.* (1998) [14]	121	28%	11	37
Anthracyclines vs. others	68 / 53	29 vs. 26% p < 0.001[d]	No difference	No difference
Weisenburger et al. (2000) [152]	68	42%	12	38
Anthracyclines vs. others	54 / 14	NR	No difference	No difference

CR: complete remission; EFS: event-free survival; PFS: progression-free survival; OS: overall survival; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone; COP: cyclophosphamide, vincristine, prednisone; PmM: prednimustine, mitoxantrone; EORTC: European Organization for the Research and Treatment of Cancer Lymphoma Cooperative Group; CHVmP-VB: cyclophosphamide, doxorubicin, teniposide (VM_{26}), prednisone, vincristine, bleomycin; ProMACE-MOPP: modified doxorubicin, cyclophophamide, etoposide (VP16), mechlorethamine, vincristine, procarbazine, prednisone; CVP: cyclophosphamide, vincristine, prednisone; Hyper-CVAD: cyclophosphamide, vincristine, doxorubicin, dexamethasone, high-dose methotrexate and cytarabine; SWOG: Southwest Oncology Group; RT: radiotherapy; NS: not significant; NR: not reported; FFS: failure-free survival; DFS: disease-free survival; A+: anthracycline-containing chemotherapy; ~: estimated of survival curve

[a] Overall response rates (CR + PR) 80% vs. 79%

[b] CHVmP-VB vs. ProMACE-MOPP: median PFS 21 vs. 16 months, (p = 0.04), median OS 65 vs. 33 months (p= 0.10)

[c] Previously untreated patients who received four cycles of Hyper-CVAD before stem cell transplantation

[d] The patients treated with anthracycline-containing regimen achieved significantly more frequently a CR or PR in a logistic regression analysis

Radiotherapy. Radiotherapy to treat patients with local MCL may be a good choice of treatment, although only about one fifth of the patients present with limited disease [153]. A recent preliminary report suggested that the progression-free survival of MCL patients with stage I or II disease was longer when local radiotherapy alone or with chemotherapy were given than if no radiotherapy was used [154].

High-dose therapy with stem cell transplantations

High-dose therapy with the support of stem cell transplantation (SCT) has been considered the most promising approach to improve the poor prognosis of younger patients with MCL. Encouraging response rates have been reported from high-dose chemotherapy with autologous SCT (ASCT) in MCL, the CR-rates ranging from 71 to 100% [149, 155-160], except for one study, where only 44% of the patients achieved a CR [161]. However, the long-term efficacy of ASCT is still uncertain, and exclusion of patients with a poor performance status and of patients who are unlikely to tolerate aggressive therapies is likely to produce selection bias over less aggressive treatments. The most optimistic results have shown an estimated probability of four-year survival of 80% [160], whereas in other studies the probability of three-year survival has varied from 24 to 56% [149, 161, 162]. Recently, in a preliminary report of the first prospective randomised trial by the European MCL Intergroup, a significant prolongation of event-free survival was reported in patients treated with myeloablative radiochemotherapy with ASCT support compared to patients treated with interferon α maintenance as a part of first-line treatment in chemosensitive patients with MCL [163]. However, there was, as yet, no difference in OS.

Contamination of the autograft by tumour cells has been considered a considerable problem in ASCT in lymphomas with bone marrow involvement [164]. Whether ex vivo manipulation such as CD34+ selection of the autograft could improve the outcome of patients with MCL after ASCT is unclear. Effective purging of the graft seems, nevertheless, to be difficult in MCL: recently in a study of 19 patients with PCR-detectable residual disease in bone marrow before high-dose therapy, eradication of the MCL was achieved in only two cases after immunological purging of the graft, and reinfusion of minimal residual disease was found to be associated with a poor outcome [165].

Allogeneic SCT has been demonstrated to be effective in MCL. The advantages of this technique include a tumour-free graft and a proposed graft-versus-lymphoma effect [166-169]. In a series including 32 patients with poor risk MCL (88% of patients beyond first remission) treated with high-dose chemotherapy or fludarabine-based nonablative combination therapy followed by allogeneic SCT, the overall four-year disease-free survival rate was 41%; and for patients transplanted in CR the figure was 100% [170].

Immunotherapy

Various immunotherapies have been found to be promising in the treatment of lymphomas. Of these, rituximab, a chimeric monoclonal anti-CD20 antibody has been studied most widely [171]. Its anti-lymphoma activity was primarily demonstrated in the treatment of follicular lymphoma patients who had received prior chemotherapy [172, 173]. In MCL the response rates have ranged from 22 to 40% when rituximab has been used as a single agent. The proportions of responding patients are rather similar among newly diagnosed and previously treated patients, and comparable to those seen among patients with follicular lymphoma: the median duration of response has been around one year [174-176]. Although these results might suggest that rituximab monotherapy does not offer higher efficacy than cytotoxic chemotherapy, the combination of rituximab and chemotherapy has been associated with a high rate of molecular response in the bone marrow and the peripheral blood [177]. The use of rituximab for in vivo purging in conjunction with high-dose chemotherapy followed by ASCT is under investigation.

Factors related to outcome

Many molecular and clinicopathological variables have been investigated in retrospective studies to examine their prognostic and predictive value with regard to the outcome of patients with MCL. However, the number of patients included in most of these studies has been limited which has hampered the identification of factors with independent influence on outcome.

Tumour-related factors

The blastoid morphology has been found to be associated with a poor prognosis in most studies. The median overall survival of patients with the blastoid variant of MCL has been less than 24 months in most reported series [7, 10-13]. More conflicting results have been reported concerning the association between the different growth patterns of MCL and outcome. Although long survival times have been found to be associated with the mantle zone pattern in some studies [100, 114], this has not been the case in all studies [9, 10, 14, 152].

p53 gene mutations or overexpression of the p53 protein are associated with a poor outcome, but these changes are also closely related to the blastoid morphology [15-18]. Similarly, enhanced cell proliferation has been shown to be associated with the blastoid morphology and with a poor prognosis [10, 18, 113, 178]. Interestingly, the mitotic index, but not the blastoid morphology, was found to be an independent prognostic factor in a multivariate analysis in one study [11]. In addition, loss of the expression of CDK inhibitor p27 has been reported to associate with decreased overall survival [18].

Patient-related factors

Many clinical factors predict the outcome of patients with MCL. Of these, advanced age, Ann Arbor stage III or IV, a poor performance status (PS), peripheral blood involvement, splenomegaly, and an elevated serum lactate dehydrogenase level (LDH) have been found most frequently to associate with an unfavourable outcome [9-11, 14, 21, 115]. However, the reported factors and their independency as prognostic factors vary from one study to another. Table 3 summarises the adverse prognostic factors for survival identified in studies with more than 50 MCL patients.

The International Prognostic Index (IPI). The IPI was originally proposed for predicting the outcome of patients with the large cell diffuse and immunoblastic lymphomas [179], but it has later been adopted as a prognostic tool also in other types of NHL [180]. The IPI categorises patients into four risk-groups: low, low-intermediate, high-intermediate, and high-risk group according to five clinical variables, i.e. age (≤ 60 years vs. > 60 years), Ann Arbor stage (I - II vs. III - IV), WHO performance status (0 - 1 vs. 2 - 4), number of extranodal sites of the disease (0 - 1 vs. >1), and level of serum LDH (normal vs. elevated). The association of high or high-intermediate risk group with a poor outcome has been shown in some studies of patients with MCL [11, 14, 21, 115, 152], but on the other hand, in other studies no significant association with outcome or only a small subgroup of patients with low risk was identified [10, 113]. These controversial results may, at least partly, be explained by the small number of patients in these studies.

Table 3. *Statistical significance of 16 clinical factors in predicting adverse overall survival in patients with mantle cell lymphoma. Only series with more than 50 patients are included. Factors found to have independent prognostic significance by multivariate analysis are marked with an asterisk.*

Variable	Norton *et al.* (1995) [9]	Zucca *et al.* (1995) [21]	Argatoff *et al.* (1997) [10][a]	Bosch *et al.* (1998) [11]	Samaha *et al.* (1998) [14]	Andersen et *al.* (2002) [115]
N of patients	66	65	80	59	121	105
Median follow-up time (months)	-	49	39	29	-	30
Median OS (months)	36	42	43	49	37	30
Age > 60	.01[b]	.003*	NS	NS	.008[b,]*	<.006[c]
Poor PS (WHO ≥ 2)	-	NS	<.01	.001*	.001*	<.001*
Advanced stage	.01*	NS	NS	NS	.02	<.02
B symptoms present	-	NS	-	.05	NS	NS
BM involvement	.005	NS	-	NS	NS	NS
Leukaemic disease	.002	NS	-	NS	.002*	-
Bulky disease	-	-	-	NS	NS*	-
High IPI	-	.003	-	.042	.02	<.001
Splenomegaly	.004*	NS	-	.012*	.04	<.001*
Extranodal sites ≥ 2	-	NS	NS	NS	<.001	NS
Anaemia	-	-	-	-	.014*	<.001*
Leukocytosis	-	-	-	.049	-	0.02
Elevated LDH	-	.001*	NS	.027	.009	0.002
Elevated serum β2 microglobulin	-	.008	-	-	.04	-
Low serum sodium	.001	-	-	-	-	-
Low serum albumin	< .001*	-	-	-	NS	NS

OS: overall survival; PS: performance status; BM: bone marrow; IPI: the International Prognostic Index; LDH: serum lactate dehydrogenase; NS: no significant effect on survival; -: not reported

[a] Multivariate analysis was not performed
[b] Reported for over 70 years of age
[c] Reported for over 65 years of age

AIMS OF THE STUDY

The purpose of this thesis was to analyse the clinicopathological features and outcome of patients with MCL, and to examine the prognostic value of different clinicopathological factors.

The specific aims were as follows:

- To study the presenting clinical and histopathological features and their prognostic significance in patients with MCL. In particular, the role of the International Prognostic Index (IPI) in predicting the prognosis in MCL was evaluated (study I and IV).

- To study the incidence, clinical characteristics, and outcome of patients with MCL and CNS involvement (study II).

- To study the incidence of blastoid transformation during the course of the disease, the related clinical characteristics and outcome, and which factors predict transformation (study V).

- To study DNA copy number changes and their clinical significance in MCL (study III).

- To analyse the effect of high-dose chemotherapy supported by autologous stem cell transplantation to treat MCL (study VI).

PATIENTS, MATERIALS, AND METHODS

Patients

The patients were identified from the database of the Department of Pathology, Helsinki University Central Hospital (studies I-V). The histological sections of all consecutive patients originally diagnosed with lymphoma of the diffuse centrocytic type (according to the Kiel classification), or the mantle cell type (according to the REAL classification) from November 1980 to September 1999 were re-examined (K.F.). Only cases fulfilling the morphological criteria of MCL according to the REAL / WHO classification [2, 3] were included in the studies. At the time of re-examination additional immunohistochemical studies were performed, if the immunophenotyping performed at diagnosis was insufficient. In some of the older cases diagnosed before the antibodies to cyclin D1 and CD5 were available, no paraffine embedded or frozen material was available for additional stainings (n = 12). However, the morphological features as well as other immunohistochemical studies were typical of MCL in all these cases.

In addition, the hospital records of all consecutive patients with confirmed MCL (according to the REAL criteria), who were scheduled for ASCT in any of the five Finnish transplant centres from March 1995 to September 2000 were collected (study VI).

The immunophenotypical findings are presented in Table 4.

Study I and II. The clinical features, treatment, and prognosis of 94 patients diagnosed with MCL between November 1980 and April 1996 were retrospectively analysed. The median follow-up time was 78 months (range 6-198 months) for all patients and 51 months (range 6-129 months) for the surviving patients. The follow-up times were computed in this and the other studies from the date of the diagnosis of MCL to the closing date of the study. Study II examined the incidence, clinical characteristics, and outcome of CNS involvement of these patients.

Study III. Thirty-four tumour specimens obtained from the 27 patients included in study I were taken for a comparative genomic hybridisation (CGH) analysis. The specimens were obtained from consecutive patients with available study material, prioritising patients with recurrent tissue samples or blastoid variant of MCL. In addition to the primary tumour, there were four cases where one to two relapses could be examined. The clinical characteristics of this cohort of 27 patients were comparable to the entire series. At the time of diagnosis, the median age of the patients was 62 years (range 45 - 80 years), and 16 (59%) were men. Two patients had stage I disease at

diagnosis, three stage II, and 22 stage IV. Twelve out of the 34 samples were classified as blastoid MCL. Twenty-one specimens were obtained from untreated lymphoma at diagnosis, three from persisting lymphoma after primary treatment and ten from relapsed lymphoma. Specimens were obtained from a lymph node (n = 23), blood (n = 3, never the only specimen), bone marrow (n = 2), spleen (n = 2), subcutaneous tissue (n = 2), tonsil (n = 1), or the mesentery (n = 1).

Study IV and V. The hospital records and histopathological material of 127 patients diagnosed between November 1980 and September 1999 were reviewed to assess the prognostic significance of the histopathological features of MCL. The cell proliferation rate was assessed by Ki-67 immunostaining and mitosis counting. In addition, overexpression of the p53 protein was studied immunohistochemically. Paraffin-embedded material for immunohistochemical staining was available in 81 cases. In addition, immunostaining for Ki-67 had been done at the time of the diagnosis in 15 cases and was available for re-evaluation. The median follow-up time was 87 months (range 7 - 238 months) for all patients and 44 months (range 7 - 169 months) for the alive patients.

Of the total of 127 patients, 52 presented with the common variant MCL and had one or more rebiopsies taken during the course of the disease. The incidence of blastoid transformation during the course of the disease and the factors predictive for lymphoma transformation were analysed (study V). The median follow-up time was 85 months (range 10 - 238 months) for all patients, and 65 months (range 24 - 153) for those alive.

Study VI. The results of peripheral blood stem cell mobilisation, response to high-dose chemotherapy, and outcome of 48 patients scheduled for ASCT treatment between March 1995 and September 2000 were retrospectively analysed. The median follow-up time of all patients was 42 months (range 14 - 164 months). At diagnosis, the median age was 56 years (range 31 - 69 years), and 40% of the patients were over 60 at the time of stem cell mobilisation. Thirty-eight patients had MCL with the typical histological features of MCL, and ten patients had the blastoid variant. Thirty-one patients (65%) were scheduled for ASCT as part of the first-line treatment, six of the second-line treatment, and 11 were treated after a relapse. A median of five (range 2 - 18) prior chemotherapy cycles had been given before mobilisation. None of the patients had progressive chemoresistant disease at the time of stem cell mobilisation.

Evaluation of clinical features (studies I-VI)

The clinical features recorded at the time of the diagnosis included age, gender, PS, Ann Arbor stage [181], presence of B symptoms, the IPI [179], diameter of the largest tumour, sites of lymphomatous involvement, number of extranodal disease sites, and blood cell counts and blood chemistry. Chest X-ray, computed tomography of the chest, abdomen, and pelvis, and a bone marrow aspirate and biopsy were performed as staging

Table 4. *Immunophenotypes of the MCLs. Immunohistochemistry (from paraffin-embedded and/or frozen tissue material), and/or flow cytometry were used for phenotyping.*

Antigen	Study I-II[a]	Study III	Study IV	Study V	Study VI
CD20 or CD19 positive	94/94 (100%)	27/27 (100%)	127/127 (100%)	52/52 (100%)	48/48 (100%)
CD3 or CD2 negative	94/94 (100%)	27/27 (100%)	127/127 (100%)	52/52 (100%)	48/48 (100%)
CD5 positive	70/82 (85%)	26/27 (96%)	102/113 (90%)	42/47 (89%)	34/36 (94%)
IgM positive	64/64 (100%)	25/25 (100%)	72/73 (99%)	35/35 (100%)	13/14 (93%)
IgD positive	48/57 (84%)	21/24 (88%)	51/64 (80%)	29/32 (91%)	10/15 (67%)
Kappa clonality	34/71 (48%)	11/25 (44%)	38/87 (44%)	18/42 (43%)	8/25 (32%)
Lambda clonality	37/71 (52%)	14/25 (56%)	49/87 (56%)	24/42 (57%)	17/25 (68%)
Cyclin D1 positive	60/60 (100%)	25/27 (93%)	105/115 (91%)	45/48 (94%)	26/27 (96%)

[a] The lymphomas of all four patients who developed CNS involvement (study II) were CD5 positive and cyclin D1 positive

examinations. Involvement of extranodal sites was considered certain when confirmed by a biopsy. In study II, the diagnosis of CNS involvement was based on clinical findings and on the presence of malignant cells with a morphology consistent with MCL in the cerebrospinal fluid. Computed tomography or magnetic resonance imaging of the brain were done to detect parenchymal or meningeal infiltrations. Epidural tumour was not considered to be a CNS relapse. In study VI, tumour re-staging including bone marrow examination (if previously positive) was evaluated shortly before the start of the mobilisation therapy and before the conditioning therapy.

Evaluation of morphological variants (studies IV-V)

The histological biopsies taken at the time of the diagnosis and, when available, at the time of disease progression were reviewed and reclassified according to the cytomorphological variant and the architectural pattern of the lymphoma. This was performed by an experienced pathologist (K.F.) who had no knowledge of survival data. The diagnostic biopsy was taken from a lymph node (n = 86), pharyngeal tonsil (n = 17), gastrointestinal tract (n = 12), bone marrow (n = 6), or from another extranodal site (n = 6). At the time of disease recurrence or progression one or more histological rebiopsies were taken of 40 patients (lymph node n = 21, tonsil n = 3, gastrointestinal tract n = 5, spleen n = 3, bone marrow n = 28, other extranodal site n = 6) .

Cytological variant. The lymphomas were divided into the two cytological variants, the common variant and the blastoid variant, as previously described (REAL/WHO classification) [2, 3]. The cases with either the lymphoblastoid or pleomorphic morphology were included into the blastoid variant. The common variant was characterised by the presence of tumour cells typical of MCL consisting of small to medium-sized lymphoid cells with slightly irregular or cleaved nuclei. The lymphoblastoid variant was characterised by tumour cells with medium sized nuclei, small nucleoli, fine chromatin, and a scant non-basophilic cytoplasm. In the pleomorphic cases variation in the size and shape of the individual lymphoma cells from medium to large size, with coarse chromatin in some cases, were seen. Cases with only rare single cells or small groups of cells that resembled the larger cells in the blastoid variant of MCL were included in the common variant.

In addition to the histological rebiopsies available in 40 patients, the cytological variant at the time of disease recurrence or progression could be assessed in an additional 12 patients from cytological specimens (bone marrow aspirate / peripheral blood smears). Morphological analysis of these samples was performed using May-Grünwald-Giemsa staining. The proportion of cells with the blastoid appearance was counted in the lymphoid infiltrates of the bone marrow aspirates. The specimen was defined as blastoid MCL if more than 30% of the lymphoid cells had a blastoid morphology. A large-sized cell was considered blastoid if it had a small to moderate amount of pale or slightly

basophilic cytoplasm and a fine or only minimally condensed nuclear chromatin. One thousand cells in each specimen were evaluated.

Architectural pattern. Three architectural patterns were defined: mantle zone (MZ) type, nodular type, and diffuse type. The lymphoma was of the mantle zone pattern when more than one half of the section area consisted of broad mantle zones of lymphoma cells, giving the surrounding germinal centres a nodular appearance. In the nodular pattern more than half of the section area contained tumour cells organised into nodules, somewhat resembling the structure of follicular lymphoma. The remaining MCLs were classified as diffuse. The architectural pattern was not evaluated in samples obtained from the bone marrow or in other extranodal biopsies if the biopsy was of an insufficient size for reliable classification.

Mitotic index. A mitotic score was counted from 10 high power fields (hpf) (ocular 10 x, non wide field view, and objective 40 x, the surface area of a hpf was 0.2 mm^2). The proliferative activity was not evaluated from bone marrow biopsies.

Immunohistochemistry (studies I-VI)

Antibodies. Immunohistochemical staining for cyclin D1 (antibody cyclin D1-GM, Novocastra Laboratories Ltd., Newcastle, UK) was performed when paraffin-embedded material was available, unless this had already been done at the time of diagnosis. The monoclonal antibody DO-7 (DAKO, Glostrup, Denmark) was used for detection of p53 protein overexpression, and a polyclonal antibody detecting the Ki-67 antigen (DAKO) for identification of the cycling cells. The antibody for Ki-67 detects a protein expressed during the G1, S, G2, and M-phase of the cell cycle and is a widely accepted method for assessing the cell proliferation rate.

Immunoperoxidase staining. Formalin-fixed deparaffinised tissue sections (4 μm) were used for the immunostainings. A standard streptavidin-biotin peroxidase technique was used after 15 minutes of microwave pre-treatment in 10 mmol/litre citrate buffer (pH 6.0). The deparaffinised tissue sections were incubated with the primary antibodies (dilution 1:25 for cyclin D1, 1:50 for p53, and 1:200 for Ki-67) overnight at 4°C. The sections were developed in 3-amino-9-ethylcarbazole (AEC). Finally, the sections were counterstained in Mayer's hematoxylin and mounted in an aqueous mounting medium. A positive control with known antigen expression was included in each experiment.

Interpretation of immunostaining results. The specimen was classified as positive for p53 overexpression when 5% or more of the lymphoma cell nuclei stained positively. The level of Ki-67 expression was assessed using a point-counting ocular grid (R.R.). At least 200 grid cross-sections falling over the lymphoma cells were evaluated, and the number of cells expressing Ki-67 and located at the grid cross-sections was counted. To simulate

routine clinical circumstances, the Ki-67 expression level was also evaluated by another investigator (K.F.) who was blinded to the counting results. The results obtained by grid counting and simple estimation of the percentage of Ki-67 positive cells correlated strongly ($r = 0.775$, $p < 0.001$).

Genetic studies (study III)

Cytogenetic analysis

Conventional karyotype analysis was performed routinely in 22 cases (18 patients) at the time of diagnosis or recurrence, as described elsewhere [77].

Fluorescence in situ hybridisation (FISH)

Samples from 11 patients were analysed by FISH on archival, Entellan-covered metaphase preparations by using a chromosome 11-specific probe to identify overrepresentation of the chromosome or the presence of translocation. After pretreatment including soaking in xylene the slides were immersed through ethanol series, fixed in a cold (4 C°) buffered formaldehyde-acetone mixture, washed, and air dried. Then the slides were incubated two times in standard saline citrate and once in Tween 20, each for 10 min. After ethanol dehydration, in situ hybridisation was performed according to Cremer *et al*. [182]. The method has been described in a detail elsewhere [183].

Southern blot hybridisation

BCL1 rearrangement was analysed in 23 cases using standard Southern blot hybridisation, as described elsewhere [77]. In short, DNA (7.5 μg) was digested with *Bam*HI restriction endonuclease (New England Biolabs Ltd., Hitchin, UK), and the fragments were size fractionated in a 0.8% agarose gel. After transfer of the fragments onto a nitrocellulose filter (Amersham, Aylesbury, UK), the DNA was hybridised with the *BCL1* probe for the MTC region. The probe was labelled radioactively with $\alpha[^{32}P]$ deoxycytidine triphosphate by using nick-translation. A sample with a known *BCL1* rearrangement was used as a positive control in all experiments.

Comparative genomic hybridisation (CGH)

Comparative genomic hybridisation (CGH) was used to evaluate DNA copy number changes in MCL. CGH was performed as described by Kallioniemi *et al.* [184, 185], with slight modifications. One microgram of DNA extracted from the lymphoma (fresh frozen tissue, blood, or bone marrow in 31 cases, and paraffine-embedded material in three cases) and reference DNA extracted from the blood of a healthy donor (same sex as the patient) were used for the hybridisation. The tumour DNA was labelled with fluorescein-12-dUTP, and the reference DNA was conjugated to Texas red R-5-dUTP (DuPont NEN Products, Boston, MA, USA). Ten micrograms of human Cot-1-DNA

were used to block the binding of the repetitive DNA sequences. The results were analysed by using an Olympus fluorescence microscope (Tokyo, Japan) and an automated CGH software package (MetaSystems GmbH, Altlussheim, Germany). The cut-off value was 0.85 for DNA copy number losses and 1.17 for gains. If the profiles exceeded the cut-off value 1.5, the region was considered highly amplified. Ninety-nine percent confidence limits with 1% error probability were used to confirm the interpretation. The cut-off values were taken from negative control experiments by using differentially labelled male DNA vs. female DNA. In the negative controls, the profiles never exceeded these limits. Positive controls with known aberrations were also set up for each hybridisation. Chromosomes 19 and Y were excluded from the analysis because of the false positive results in negative controls.

Loss of heterozygosity (LOH) analysis

LOH analysis was performed in seven lymphomas to study 11q21-24 deletion using polymorphic microsatellite markers in patients from whom it was possible to obtain reference DNA from either the blood or the bone marrow or from other unaffected tissue. Thirteen polymorphic markers were selected, and they covered the region from 11q21 to 11q24, according to the Entrez database (National Center for Biotechnology Information, NIH, Bethesda, MD, USA). The markers spanned a region of 33 cM with an average interval of 2.8 cM [186]. The used markers were D11S900, D11S1339, D11S1325, D11S1343, D11S938, D11S939, D11S1341, D11S1356, D11S1336, D11S1353, D11S933 (Genosys, Cambridge, UK), D11S1347, and D11S934 (Généthon, Paris, France). LOH analysis was performed as described previously [187]. Fifty nanograms of DNA were used for each PCR reaction (amplified in 27 cycles in a volume of 10 μl). The PCR products were electrophoresed in a 6% polyacrylamide gel, dried, and exposed to Kodak XAR film. Patient samples were designated uninformative when homozygous and informative when heterozygous.

Treatment of lymphoma (studies I-V)

The first-line treatments are presented in Table 5. Of the total of 127 patients, six patients did not receive active treatment for their lymphoma. Most of the patients achieved moderately intensive, usually anthracycline-containing chemotherapy (n = 84). Seven additional patients underwent autologous (n = 4) or allogeneic (n = 3) SCT as the first-line treatment after having received 3 to 7 cycles of CHOP. Less aggressive chemotherapy with chlorambucil (with or without prednisone) or CVP was given to 24 patients. Six of the 22 patients with stage I or II disease were operated on or treated with local radiotherapy only. Patients without a satisfactory response to the first-line therapy were given various additional chemotherapy regimes and/or radiotherapy.

Table 5. *First-line therapies.*

Treatment	Study I-II[a] (n = 94)	Study III (n = 27)	Study IV (n = 127)	Study V (n = 52)
No treatment	5	2	6	0
Surgery	5	0	4	0
Radiotherapy	3	1	2	1
Chlorambucil ± prednisone	13	5	16	7
CVP	6	1	8	3
CHOP / CNOP	32	4	51[b]	18
M-BACOD	27	12	27	16
ESHAP	3	1	9	5
Others[c]	0	1	4	2

CVP: cyclophosphamide, vincristine, prednisone; M-BACOD: high-dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone with or without bleomycin; CNOP: as CHOP but mitoxantrone in place of doxorubicin; ESHAP: etoposide, methylprednisolone, cytarabine, cisplatin

[a] Of the four patients who developed CNS involvement (study II), three received M-BACOD and one chlorambucil as the first-line treatment
[b] Includes 7 patients who underwent autologous (n = 4) or allogeneic (n = 3) stem cell transplantation as part of the first-line treatment after 3 to 7 cycles of CHOP
[c] Anthracycline-containing regimens

High-dose chemotherapy with autologous stem cell transplantation (ASCT) (study VI)

Treatment before stem cell mobilisation. Patients with mobilisation during the first-line treatment (n = 31) had received a median of 3 (range 2 - 10) prior cycles of CHOP or a CHOP-like regimen (n= 3) with methotrexate or etoposide. Two or more regimens (a median of 7 prior cycles) had been given to the patients who underwent stem cell mobilisation during the second-line treatment (n = 6), and to the patients who underwent mobilisation (a median of 12 cycles) after relapse (n = 11).

Mobilisation and conditioning regimens. The chemotherapy regimens used for peripheral blood stem cell (PBSC) mobilisation were: cyclophosphamide 4000 mg/m^2 (n = 29), intermediate-dose-CHOP (cyclophosphamide 1200 mg/m^2, doxorubicin 75 mg/m^2, vincristine 2 mg, prednisone 100 mg/m^2, n = 14), or others (n = 5). All patients received granulocyte colony-stimulating factor (G-CSF) 5 or 10 μg/kg s.c.

The conditioning regimens were: BEAM (BCNU, etoposide, cytarabine and melphalan,

n = 17), BEAC (BCNU, etoposide, cytarabine, and cyclophosphamide, n = 14), and total-body irradiation (TBI) 12 Grays (Gy) with cyclophosphamide (n = 4). G-CSF was given to 33 patients after intensive therapy. After transplantation, eight patients received local radiotherapy (n = 7) or rituximab (n = 1) for consolidation.

Assessment of response and survival (studies I-VI)

Assessment of treatment response was evaluated as described by Miller *et al.* [188]. In short, complete response (CR) was defined as the total disappearance of all clinical evidence of the disease, including normalisation of the radiographic results and the bone marrow biopsy if abnormal before treatment. Partial response (PR) was defined as regression of at least 50% of all measurable disease. Relapse was defined as the reappearance of malignant lymphoma in a patient who had previously been in complete remission. Progression was defined as relapse, increase of tumour size by 25%, or appearance of a new tumour. The duration of remission was defined as the time from the documentation of a CR to relapse. Time to treatment failure (TTF) was measured from the date of diagnosis to the progression of lymphoma, or to death from any cause [189]. Overall survival (OS) was the time interval between the date of diagnosis and death. In study V, the time intervals between the diagnosis and the date of rebiopsy, and survival after rebiopsy were calculated. In study VI, the event-free survival (EFS) was measured from the date of transplantation to the date of progression, or to death from any cause. Progression-free survival (PFS) was defined as survival from the date of transplantation to the date of lymphoma progression. The patients who did not exhibit progression of the lymphoma were censored at the time of last date follow-up when TTF, EFS or PFS were calculated. Similarly, the patients who were alive at the time of the last follow-up were censored when OS was calculated.

Statistical methods (studies I-VI)

The statistical analyses were performed on a VAX 6000 computer using the BMDP statistical software package (studies I-III), or using the SPSS statistical program 9.0 for Windows (studies IV-VI). Frequency tables in all studies were analysed using the chi-square test or Fisher's exact test. Mann-Whitney U-test was used to compare the non-normal distributions between two groups, and Spearman’s test to examine the correlation between continuous variables. All survival times were estimated using the method of Kaplan and Meier. The univariate survival analyses were performed using the Mantle-Cox and Wilcoxon tests. The relative importance of prognostic factors was analysed using Cox’s proportional hazards regression analysis.

All p-values are two-sided.

RESULTS

CLINICAL, HISTOPATHOLOGICAL, AND GENETIC FEATURES

Clinical features at diagnosis (study I, IV)

The median age of all 127 MCL patients was 65 years (range 30 - 90 years) at the time of diagnosis. Sixty-four percent were males. Although 83% of the patients had stage III or IV disease, the PS was usually good (WHO 0 - 1 in 85%), and only one third of the patients had B symptoms. Bulky tumours were rare, but 44% of the patients presented with more than one extranodal site of involvement. The most typical extranodal site was the bone marrow, and bone marrow infiltration occurred in 63% of the patients. The spleen, the gastrointestinal tract, and Waldeyer's ring were other common sites of lymphoma involvement. Lymphoma cells were present in the peripheral blood of only 15% of the patients. None of the patients presented with the CNS involvement. The patients were almost equally distributed among the four risk groups according to the IPI. The clinical characteristics of the patients are summarised in Table 6.

Histopathological features at diagnosis (study IV)

Histological subtypes

Three various subtypes of MCL were defined according to the cytological variant and the architectural pattern of the lymphoma (Table 7): the mantle zone/nodular subtype of the common variant, the diffuse subtype of the common variant, and the blastoid variant, which occurred in 19% (n = 23), 64% (n = 77), and 17% (n = 20) of the cases, respectively. All cases of the blastoid variant had a diffuse growth pattern. Since the patients with the mantle zone and those with the nodular growth pattern had a similar outcome, and because of the small number of patients presenting with the mantle zone growth pattern, these patient groups were combined in further analyses.

Associations between histological subtype and clinical features. There were no significant differences in the frequency of the clinical features between the patients presenting with the mantle zone/nodular and those with the diffuse subtype of the common variant. However, as compared with the common variant of MCL, the patients with the blastoid variant were more often older than 60 years (95% vs. 70%, p = 0.019) and presented more often with B symptoms (55% vs. 31%, p = 0.046), had more often bone marrow and blood involvement (90% vs. 58%, p = 0.011; and 35% vs. 11%, p = 0.015, respectively), and a haemoglobin level of less than 125 g/l (79% vs. 45%, p = 0.007).

Table 6. *Clinical characteristics at the time of diagnosis.*

Variable	Study I (n = 94)	Study IV (n = 127)
Male sex	59%	64%
Age		
Median (years)	66	65
Range (years)	44 – 87	30 – 90
> 60 years	77%	74%
Stage III-IV	76%	83%
B symptoms	35%	35%
Performance status (WHO) ≤ 1	86%	85%
Largest tumour ≥ 10 cm	20%	20%
Site of involvement		
Bone marrow	61%	63%
Blood	12%	15%
Splenomegaly	31%	40%
Gastrointestinal tract	19%	20%
Conjunctiva/orbita	6%	5%
Waldeyer's ring	17%	17%
>1 extranodal site	40%	44%
International Prognostic Index		
Low	23%	15%
Low-intermediate	29%	30%
High-intermediate	27%	34%
High	22%	21%
Lactate dehydrogenase ≥ 450 U/l	38%	46%
Thymidine kinase ≥ 5 U/l	67%	80%

WHO: World Health Organization classification

Table 7. *Cytological variant and architectural pattern of 127 lymphomas at the time of diagnosis.*

Growth pattern of lymphoma	Common Variant	Blastoid variant		Total (N)
		Lympho-blastoid	Pleomorphic	
Mantle zone	2	0	0	2
Nodular	21	0	0	21
Diffuse	77	15	5	97
ND	7	0	0	7
Total (N)	107	15	5	127

ND: not definable

Proliferative activity

The median mitotic score (number of mitoses / 10 HPFs) was 5 (range 0 - 120), and the median proportion of Ki-67 expressing lymphoma cells was 20% (range 4 - 80%). As expected, there was a strong positive correlation between the mitotic score and the Ki-67 expression level ($r = 0.548$, $p < 0.001$). A higher median mitotic score and Ki-67 expression level were found in the blastoid than in the common variant of MCL, but there was no difference in the proliferation activity between lymphomas presenting with the mantle zone/nodular pattern and those presenting with the diffuse subtype of the common variant (Table 8).

Overexpression of p53 protein

The p53 protein was overexpressed in 15 (19%) of the 81 cases studied. p53 expression was seen more often in the blastoid variant (4/10, 40%) than in the common variant (11/71, 15%), but this difference was not statistically significant ($p = 0.082$) (Table 8).

Table 8. *Proliferative activity and overexpression of p53 protein according to the histological subtype in 127 MCL patients.*

Histological subtype	N of patients	Mitoses/10 HPFs Median (range) (n = 121)	Ki-67 (%) Median (range) (n = 96)	p53 positivity (n = 81)
All	127	5 (0-120)	20% (4-80%)	19%
Common variant[a]				
MZ/nodular	23	5 (0-15)	18% (7-30%)	6%
Diffuse	77	5 (0-44)	17% (4-80%)	16%
All	107	5 (0-44)[b]	17% (4-80%)[b]	15%
Blastoid variant	20	23 (13-120)[b]	40% (11-80%)[b]	40%

HPF: high power field; MZ: mantle zone

[a] The growth pattern of the lymphoma could not be defined in seven cases

[b] The difference between the common and the blastoid variant statistically significant ($p < 0.001$, Mann-Whitney's U-test)

Chromosomal features (study III)

Karyotypes

Very complex karyotypes with several marker chromosomes were seen in most of the studied 22 specimens in 18 patients. The chromosome numbers of the abnormal clone ranged from 39 to 49.

Translocation (11;14)

Of the evaluated 24 lymphomas, nine (38%) had tranlocation (11;14) or *BCL1*

rearrangement by Southern blot hybridisation (n = 7) and/or FISH (n = 4).

DNA copy number changes

DNA copy number changes were found in all 34 tumours of the 27 patients who were studied (mean 4 changes, range 1 - 11). The most common changes were gains at 3q (52%), 8q (30%), and 15q (26%), and losses at 13q (41%), 1p (33%), 6q (30%), 9p (30%), and 11q (30%). Gains and losses were detected at a similar frequency (Figure 2).

There were more DNA copy number changes in the blastoid variant than in the common variant (mean 6 vs. 3), but the difference was not statistically significant. High-level amplifications were found in five of the 12 blastoid variant cases (located at 3p14-22, 8q23-ter, 9, 12p13, 13q21-ter, 13q22-32, 15q22-ter, and 20q13.1), but in none of the common variants (n = 22). In all four patients where there were serial samples available, a higher number of changes were seen at the time of relapse as compared to the sample taken at the time of diagnosis (Table 9). Most of the changes present in the sample taken at diagnosis persisted in the sample taken at relapse.

Loss of heterozygosity at 11q. A small commonly deleted region was located at 11q22. LOH analysis covering the region from 11q21 to 11q24 was performed in seven cases, of which three lymphomas had a deletion at 11q by CGH. LOH was detected in two of these cases, but could not be identified in the third case by any of the markers. LOH was not seen in any of the four cases in which no deletion could be shown by CGH.

Figure 2. *Summary of gains and losses in MCL detected by CGH. Each line represents a change in a single tumour. The gains are on the right side of the chromosome, and losses are on the left. High-level amplifications are marked with thick bars.(Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.)*

Table 9. *DNA sequence copy number changes in four MCL patients at the time of diagnosis and at relapse.*

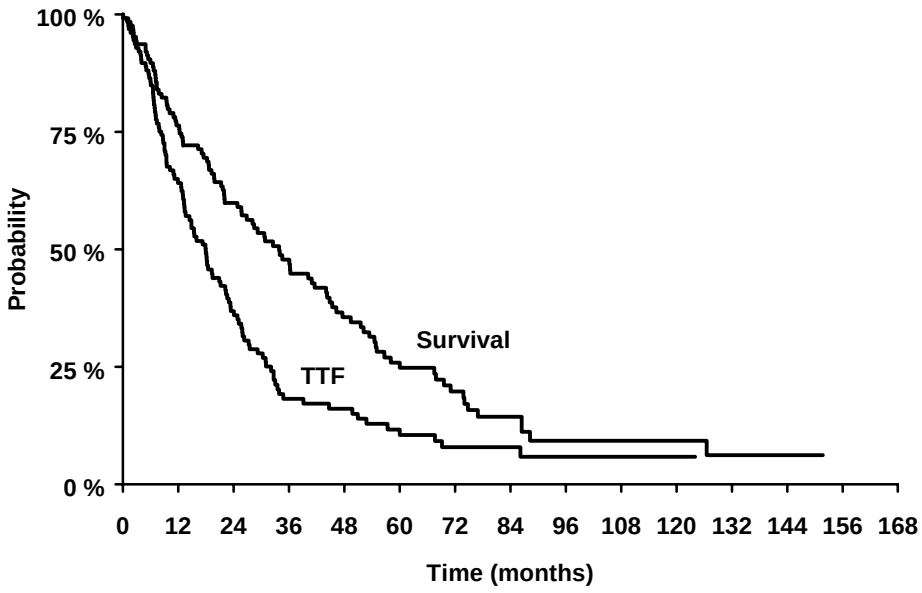
Patient no (III)	Changes at diagnosis		Changes at relapse		Time after dg (years)
	Gains	Losses	Gains	Losses	
7	3q24-29	-	a) 3q23-29, 15q21-26 b) 12q13-14, 15q21-26	a) 1p13-31 b) Xp22.1-3, 1cen-p31, 5q14-23, 7q22-36.9, 12q21-22, 18p	1 7
13	3q22-29	-	3q21-29, 8q, 11q14-22, amp 8q23-24.3	1p22-31, 5q, 6q23-27, 8p21-23, 9p24-q13, 13, 17p, 21	1
15	7p22-q22, 15q22-24	11q14-23, 15cen-q15	7p22-q22, 15q22-23	Xp, 1cen-p22, 11q14-23, 15cen-q15	5
17	4q32-35	1p13-22, 9p24-32, 11q22	4q31.3-35, 8q23-24.3	1cen-p31, 9p24-q22, 11q22, 13q21-34	6

OUTCOME AND PROGNOSTIC SIGNIFICANCE OF PRESENTING FEATURES

Outcome (study I, IV, unpublished data)

Almost one half of the actively treated patients (55/121, 45%) achieved CR, but the duration of the remissions was usually relatively short (median 16 months, 95% CI 9 - 23 months) and only 22% of the patients were in remission five years after treatment. First-line treatment produced a higher CR-rate if the treatment included an anthracyline-containing regimen or ESHAP (29%) as compared to patients who were treated with chlorambucil or CVP (13%), but the difference was not statistically significant (p = 0.127). The median TTF of all 127 patients was 18 months (95% CI 14 - 22 months) and the median OS 34 months (95% CI 27 - 41 months) (Figure 3). There was no plateau in either survival curve. The survival rate was 48% at three years and 26% at five years. The outcome of patients with cyclin D1 positive MCL (n = 105), cyclin D1 negative MCL (n = 10) and those with unknown cyclin D1 expression (n = 12) was similar.

Figure 3. *Time to treatment failure and overall survival of 127 patients with MCL.*



Prognostic factors

Clinical factors (study I, unpublished data)

Several clinical factors were prognostic of survival (Table 10, Figure 4). The most important factors by univariate analyses were advanced disease stage, presence of B

symptoms, a high IPI, bone marrow infiltration of lymphoma, leukaemic disease, a low haemoglobin level, a high blood leucocyte count, and an elevated serum LDH level at diagnosis. These variables predicted a low CR-rate, short TTF, and short OS. Advanced age (over 60 years) had no influence on the CR-rate, but was associated with short TTF and OS.

Histopathological factors (study IV)

Overexpression of p53 protein was associated with a lower CR-rate as compared to lymphomas with normal p53 expression (20% vs. 52%, respectively, $p = 0.027$), but neither the histological subtype, the mitotic score nor the level of Ki-67 expression exerted a significant effect on the likelihood of remission. Instead, all these factors were significantly predictive of TTF and OS (Table 10). The patients with the blastoid variant had the poorest outcome: the median OS was only 11 months (95% CI 9 - 14 months). There was no difference in outcome between patients with the lymphoblastoid and the pleomorphic morphology. Patients with the diffuse subtype of the common variant had a median OS of 35 months (95% CI 20 - 49 months) and those with the mantle zone/nodular subtype of the common variant of 70 months (95% CI 25 - 114 months) (Figure 5A). There was no difference in the intensity of the primary treatment between patients with different histological subtypes; 76% of the patients with the mantle zone/nodular subtype received moderately intensive chemotherapy as compared to 73% of those with the diffuse subtype of the common variant, and to 93% of the patients with the blastoid variant ($p = 0.23$).

The effects of the mitotic score and Ki-67 expression on outcome, when the highest tertile was used as the cut-off level, are shown in Table 10. The associations with survival were weaker when the median was chosen as the cut-off value instead of the highest tertile. Interestingly, the level of Ki-67 expression was found to predict survival both in the entire series and in the subset of the patients with the common variant of MCL (Figure 5B): 18 patients with the common variant and high Ki-67 ($\geq$ 26%) expression had shorter OS (median 20 months) than the 64 patients with the common variant and a low Ki-67 level ($<$ 26%, median 45 months, $p < 0.001$).

Multivariate analyses and prognostic value of the International Prognostic Index (IPI) (study I, IV)

All of the five components of the IPI except the number of disease sites (and PS on TTF), had prognostic impact on TTF and OS univariate analyses. However, only stage and LDH were significantly associated with the CR-rate, and age and stage with TTF and OS when the components of the IPI were analysed together in a multivariate analyses (study I). In study IV, a high Ki-67 expression, stage III-IV, and age over 60 years at diagnosis were found to be independent prognostic factors for OS when the histological subtype, Ki-67 expression level, and the mitotic score were analysed together with the five components of the IPI in Cox's proportional hazard regression

Table 10. *Outcome according to the clinical and histopathological features.*

Variable	N	Median TTF (months)	P	Median OS (months)	P
Total	127	18		34	
Age					
≤ 60 years	33	24		55	
> 60 years	94	14	0.019	26	0.004
Performance status					
WHO 0-1	106	18		55	
>2	19	13	0.190	28	0.040
Ann Arbor stage					
I-II	22	45		71	
III-IV	105	15	0.001	27	< 0.001
B symptoms					
Absent	81	18		40	
Present	44	13	0.003	22	< 0.001
BM involvement					
Yes	74	15		26	
No	43	25	0.004	58	< 0.001
Lymphoma cells in the blood					
Yes	19	7		13	
No	105	19	< 0.001	40	< 0.001
IPI					
Low	17	51		75	
Low-intermediate	33	22		41	
High-intermediate	38	15		31	
High	23	9	< 0.001	17	< 0.001
Haemoglobin level					
≤ 129 g/l	69	15		25	
> 129 g/l	50	25	0.006	58	< 0.001
Leucocyte count					
≤ 10 x 10^9/l	84	23		45	
> 10 x 10^9/l	34	8	< 0.001	13	< 0.001
LDH					
< 450 U/l	61	25		49	
≥ 450 U/l	52	15	0.003	22	< 0.001
Anthracycline-containing therapy					
Yes	91	19		34	
No	24	11	0.077	28	0.071
Histological subtype					
MZ/nodular, common	23	35		70	
Diffuse, common	77	18		35	
Blastoid variant	20	8	< 0.001	11	< 0.001
Mitotic score					
< 12	84	23		41	
≥ 12	40	13	0.001	13	0.001
Level of Ki-67 expression					
< 26%	65	23		45	
≥ 26%	31	9	< 0.001	13	< 0.001
p53					
Negative	66	19		41	
Positive	15	9	0.005	18	0.018

TTF: time to treatment failure; OS: overall survival; WHO: World Health Organization classification; BM: bone marrow; IPI: the International Prognostic Index; LDH: serum lactate dehydrogenase; MZ: mantle zone

Figure 4. *Overall survival by (A) age, (B) stage, (C) LDH, and (D) the IPI.*

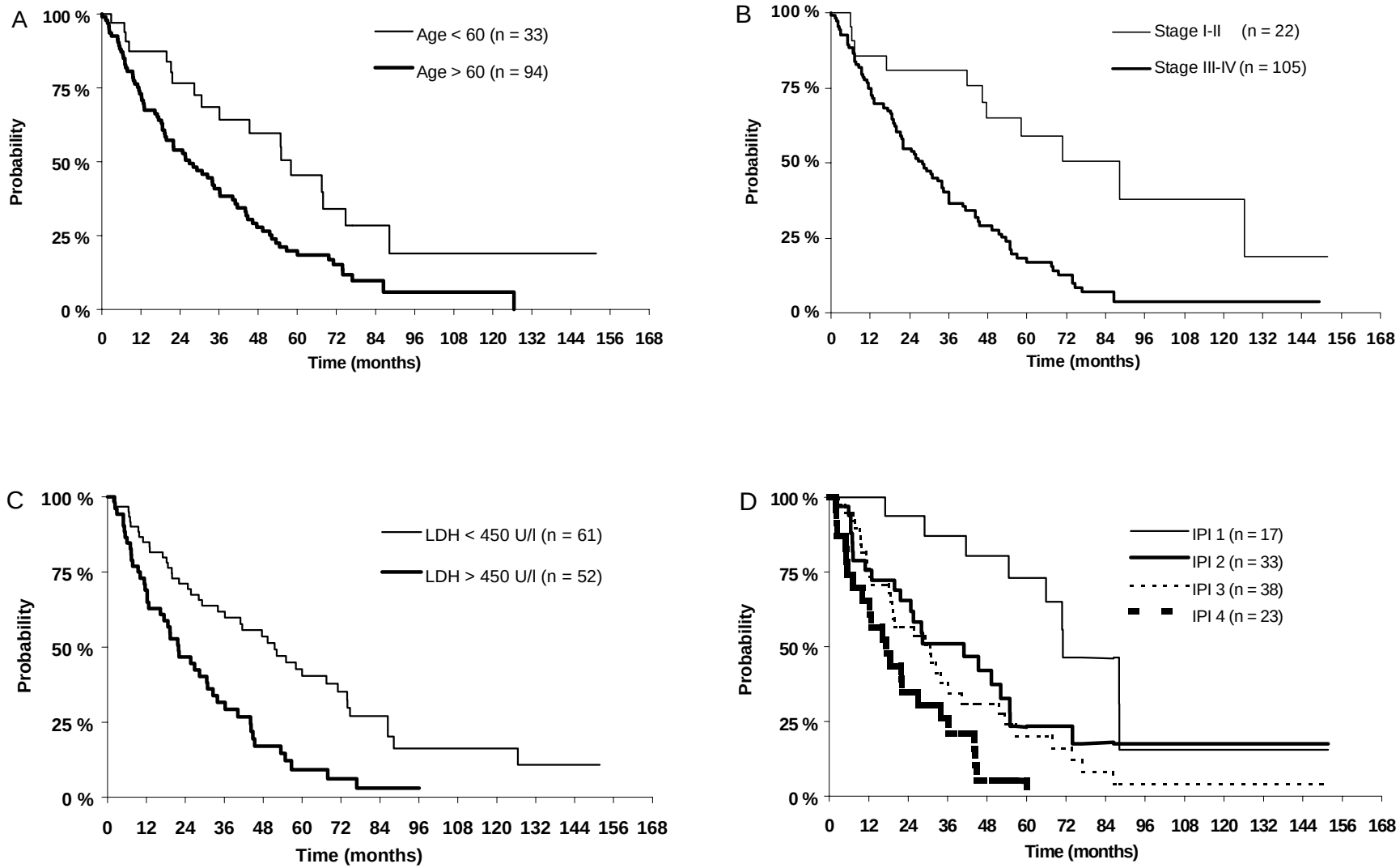
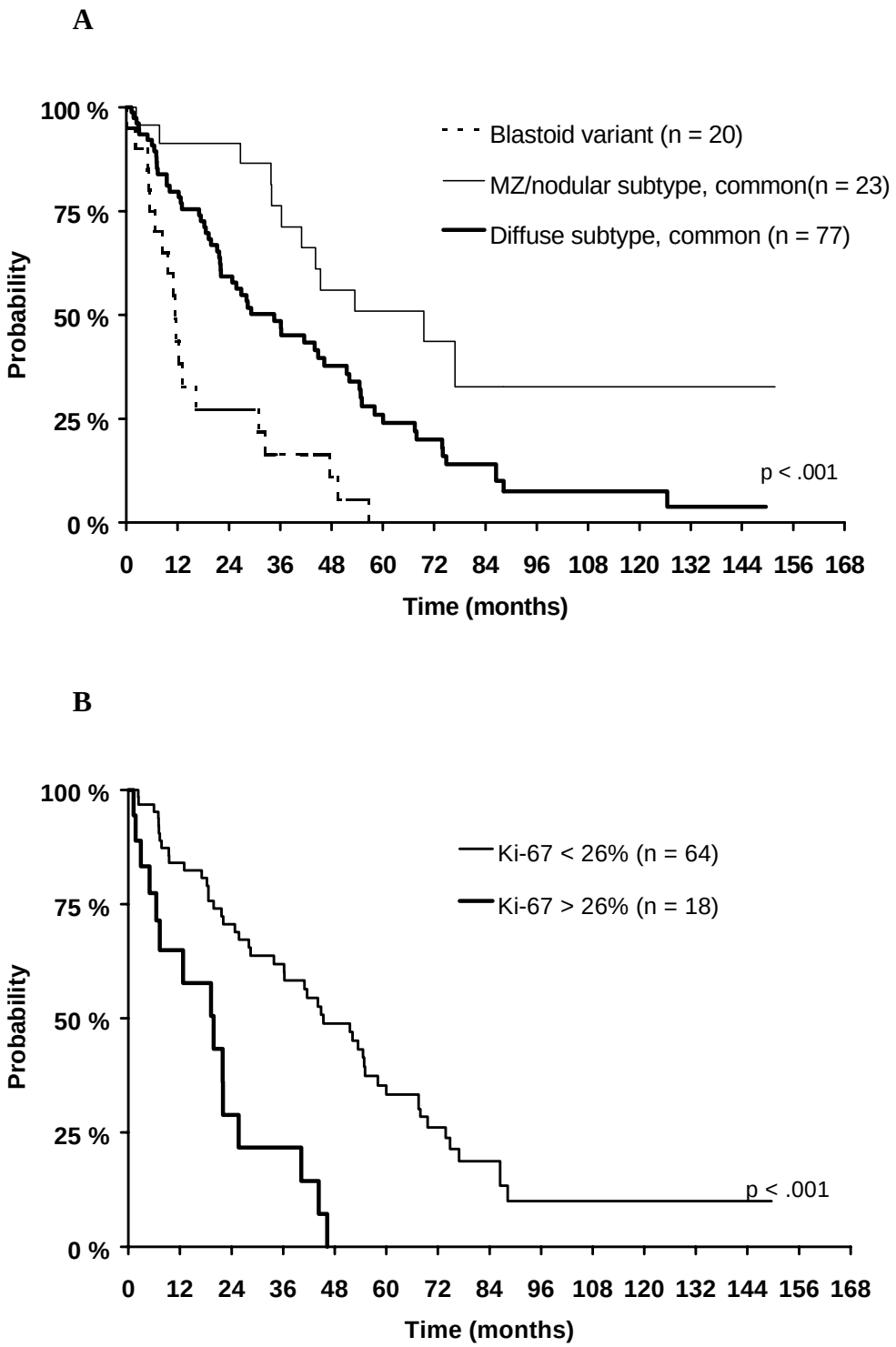


Figure 5. *Overall survival **(A)** according to the histological subtype in all patients, and **(B)** according to the Ki-67 expression level in patients with the common variant of MCL.*



model (Table 11). If the median was used as the cut-off value for the mitotic score and Ki-67 expression instead of the upper tertile, Ki-67 lost its significance, and age (RR 2.51, 95% CI 1.49 - 4.21), serum LDH level (RR 2.31, 95% CI 1.46 - 3.65), and the histological subtype (RR 2.68, 95% CI 1.39 - 5.19 for diffuse vs. the mantle zone/nodular subtype; and RR 5.35, 95% CI 2.44 - 11.79 for the blastoid variant vs. the mantle zone/nodular subtype) were retained in the model.

DNA copy number changes (unpublished data)

None of the single DNA copy number changes nor the number of changes present were associated with outcome.

Table 11. *Prognostic significance of the five IPI components and histopathological features on survival (Cox's proportional hazard regression analysis).*

Variable	Relative risk	95% CI	P
Age > 60 vs. ≤ 60 years	1.78	1.04 – 3.05	0.035
Ann Arbor stage III-IV vs. I-II	3.17	1.55 – 6.46	0.002
Performance status WHO 2-4 vs. 0-1			NS
LDH Elevated vs. normal			NS
Number of extranodal sites ≥ 2 vs. 0-1			NS
Histological subtype Blastoid vs. diffuse vs. MZ/nodular			NS
Mitoses / 10 HPFs ≥ 12 (upper tertile) vs. < 12			NS
Ki-67 expression ≥ 26% (upper tertile) vs. < 26%	3.25	1.90 – 5.58	< 0.001

CI: confidence interval; NS: not significant; WHO: World Health Organization classification; LDH: serum lactate dehydrogenase level; MZ: mantle zone; HPF: high power field

BLASTOID TRANSFORMATION (study V)

In serial samples, cytological progression from the common to the blastoid morphology occurred in 18 (35%) out of 52 patients primarily with the common variant of MCL (Figure 6). The minimum estimated risk of blastoid transformation was 24% at three, and 42% at five years. Blastoid transformation took place in 10 patients at the time of first lymphoma relapse, in one patient at first progression of refractory disease, and in seven patients during later progression of the disease. At that time, all patients had systemic

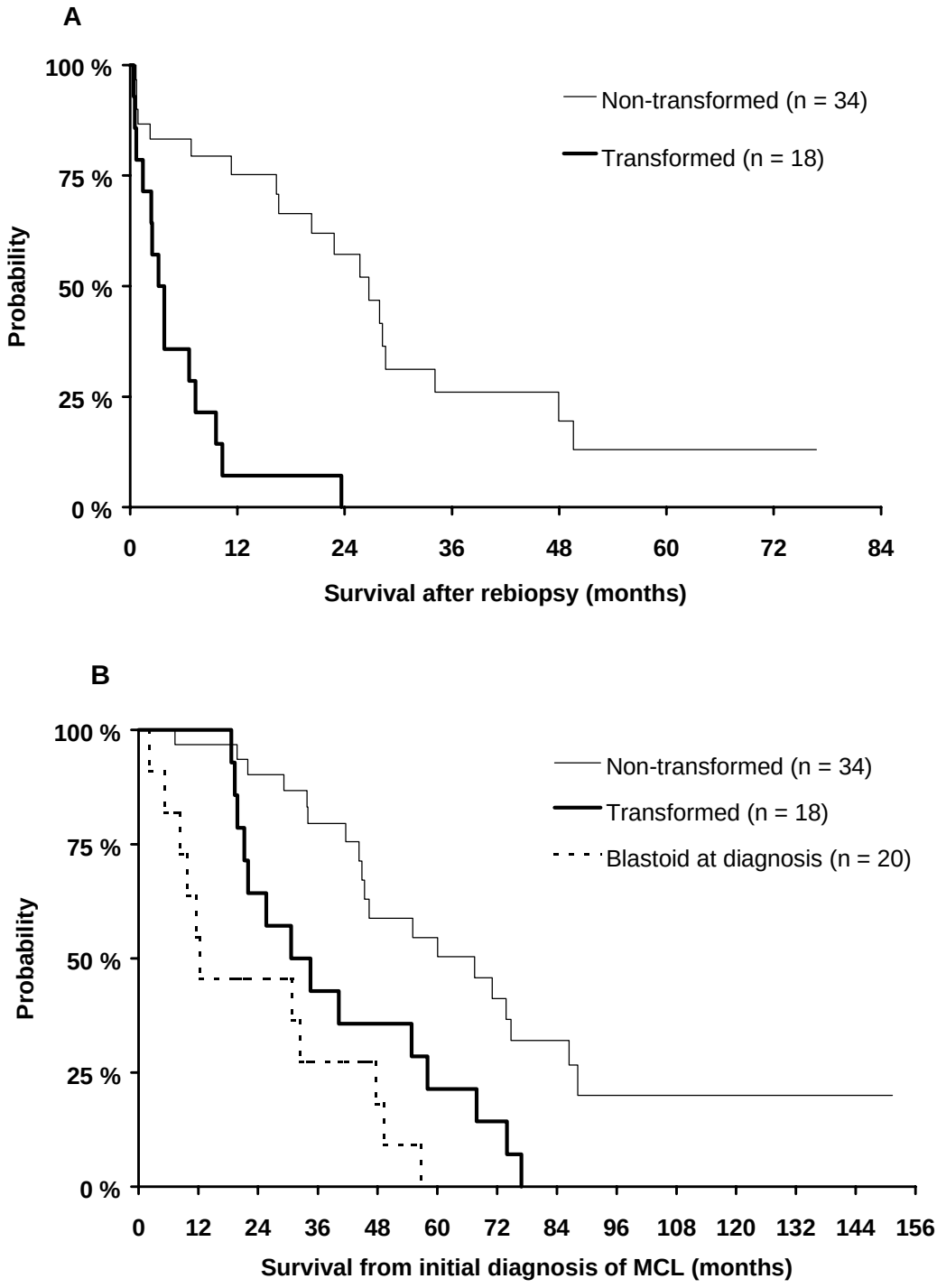
disease, and lymphatic cells with the blastoid morphology were detected in the peripheral blood in all except two patients.

Only 18% of the patients with LDH < 450 U/l at presentation developed blastoid transformation as compared with 58% of those with a higher LDH level (p = 0.004). Similarly, 28% of the patients presenting with a leucocyte count ≤ 10 x 10^9/l developed blastoid transformation as compared with 67% of the patients with leukocytosis (p = 0.049), respectively. Blastoid transformation occurred also earlier after diagnosis in patients with a high LDH level and leukocytosis (see Figures 2 and 3, study V). In addition, a higher Ki-67 expression level (median 24% vs. 17%, p = 0.023) and a higher mitotic score (median 5 vs. 3, p = 0.056) were typical of the tumours of the patients who developed blastoid transformation. Two of the three patients who had p53 overexpression at the time of diagnosis, developed blastoid transformation during the course of the disease.

The median survival time after blastoid transformation was only 3.8 months, whereas as computed from the (latest) rebiopsy it was as long as 26 months in patients without transformation (p < 0.001). The respective median OS-times from the first diagnosis of MCL were 31 and 60 months (p < 0.001) (Figure 7).

Figure 6. *Blastoid transformation of MCL. The common variant (left) and the blastoid variant (right) tumours seen in the same patient. (Hematoxylin and eosin, ×400.)*

Figure 7. ***(A)*** *Survival from the time of the rebiopsy to death of patients with blastoid transformation ("transformed") and of patients with no transformation at the time of disease progression ("non-transformed").* ***(B)*** *Survival from the initial diagnosis of the same patients. In addition, survival of 20 patients who had the blastoid variant of MCL already at the time of the primary diagnosis of MCL is shown for comparison ("blastoid at diagnosis").*



CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT (study II, V, unpublished data)

Seven (6%) of the 127 patients developed a CNS relapse within a median time of 10 months (range 5 - 86 months) after diagnosis. In five patients the CNS lymphoma related to the blastoid MCL: four of the patients presented with the blastoid variant, and in one patient the CNS lymphoma was diagnosed after blastoid transformation. Regardless of therapy with intrathecal injections of methotrexate (12.5 mg) or cytarabine (45 mg), survival after the diagnosis of CNS relapse was dismal (18 to 95 days, median 1.8 months).

RESULTS OF AUTOLOGOUS STEM CELL TRANSPLANTATION (study VI)

Stem cell mobilisation

Fourteen of the scheduled 48 patients (29%) failed to mobilise an adequate number of PBSCs (CD34+ -cells in blood > 0.020 x 10^9/l) for harvesting. Factors found to be associated significantly with mobilisation failure were a low haemoglobin level (≤ 114 g/l) at the time of mobilisation (46% vs. 16%, p = 0.042), female sex (75% vs. 20%, p = 0.005) and blastoid cytology (60% vs. 21%, p = 0.045). Only 1/11 patients (9%) in CR as compared to 13/37 non-CR patients (35%) failed to mobilise stem cells, but the difference was not statistically significant (p= 0.14, Fisher's exact test). There was no effect on the mobilisation failure by mobilisation regimen used or the dose of G-CSF (5 vs. 10 μg/kg). Nor was the number of prior chemotherapy cycles before mobilisation related to mobilisation failure, but the more cycles were given, the fewer CD34+ -cells were collected (p = 0.001).

Among 34 patients with successful mobilisation, a median of 3.8 x 10^6/kg (range 1.88 - 23.3 x 10^6/kg) CD34+ -cells was harvested with a median of two leukapheresis. In addition, after failed mobilisation, a sufficient amount of stem cells was collected from the bone marrow of one patient. CD34+ -cell selection was performed in 11 (31%) cases.

Response to transplantation

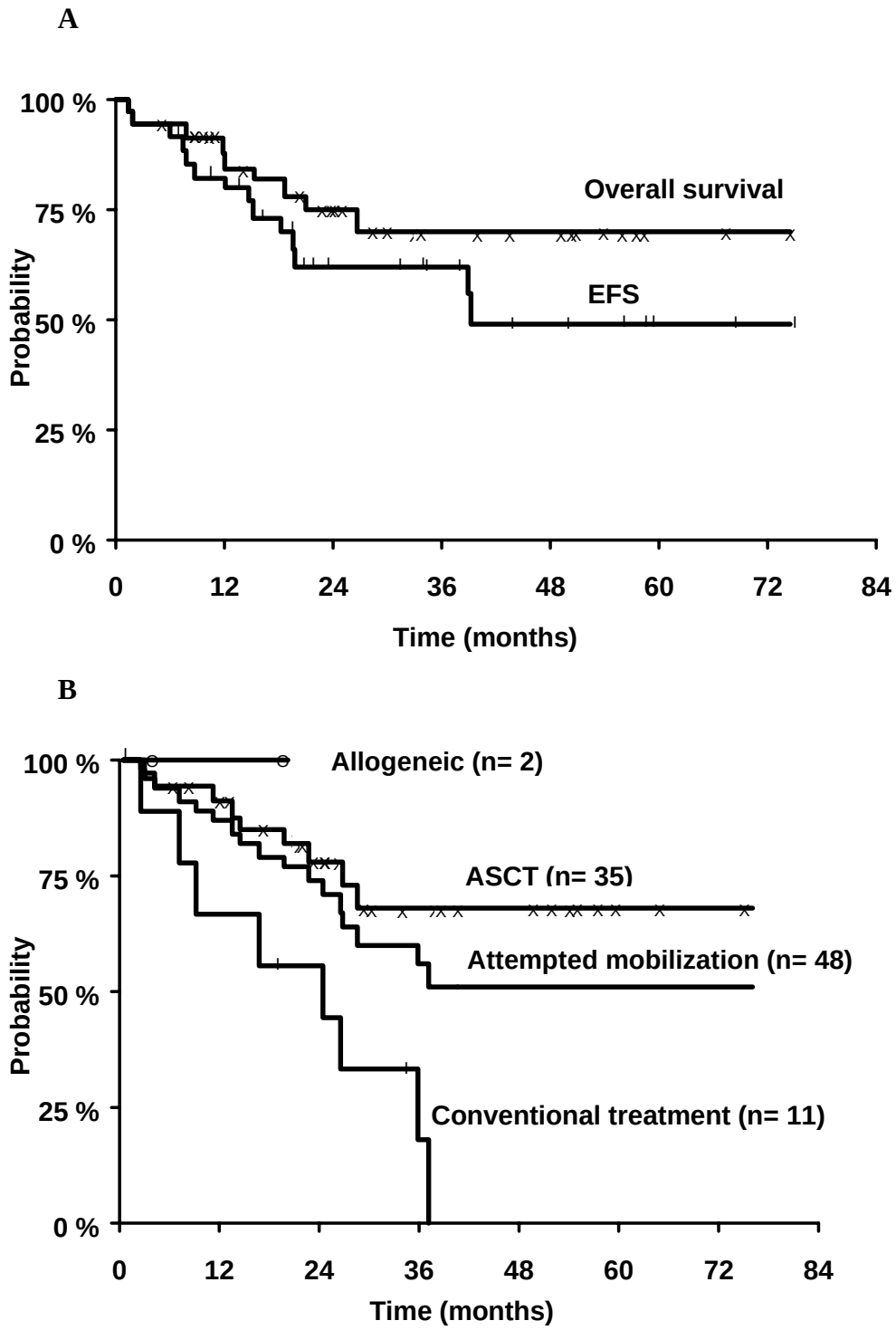
Altogether 35 patients received ASCT, 24 patients (69%) as a part of the first-line treatment, three patients during the second line treatment and eight patients after relapse. At the time of transplantation, 13 patients (37%) were in CR and 22 (63%) in PR. Twenty-eight patients (80%) remained in or achieved CR after transplantation. Of

five patients with PR (14%) after transplantation, three achieved CR later: two patients with minimal bone marrow infiltration by α-interferon, and one patient with a mediastinal tumour by radiotherapy. Two patients (5%) died of treatment-related sepsis and acute respiratory distress syndrome eight and six weeks after ASCT, respectively. At autopsy the first of these patients had no evidence of lymphoma and the second had minor bone marrow infiltration.

Outcome. During a median follow-up time of 38 months (range 9 - 75 months) after transplantation, nine (29%) of the 31 patients with CR relapsed and both patients with PR progressed. The median EFS was 39 months, and the probability of four-year PFS was 50%. The estimated four-year survival after transplantation was 69% (Figure 8A). For patients transplanted during the first-line treatment, during the second-line treatment, or after relapse, the expected four-year survival after transplantation was 62%, 100% and 75%, respectively. Splenomegaly ($p = 0.045$), gastrointestinal tract involvement ($p = 0.012$) and a high CRP level ($p < 0.001$) at diagnosis were associated with a shorter PFS, and a high CRP ($p = 0.013$) level, and bone marrow infiltration ($p = 0.045$) at diagnosis, and age over 60 years at transplantation ($p = 0.051$) with a shorter survival after transplantation. No secondary malignancies have been emerged.

Figure 8B shows the survival after attempted stem cell harvest of all 48 patients who were intended for ASCT.

Figure 8. ***(A)*** *Event-free survival (EFS) and overall survival after ASCT of 35 patients with MCL.* ***(B)*** *Survival after attempted stem cell harvest in all 48 patients who were intended for ASCT. Thirty-four patients received an autologous PBSC transplantation, and one patient with mobilisation failure received stem cells collected from the bone marrow. Of the other 13 patients who failed mobilisation of PBSCs, two received an allogeneic transplant, and the remaining 11 patients were treated with conventional therapy. (Reprinted by permission of Taylor & Francis Group.)*



DISCUSSION

Clinical presentation of mantle cell lymphoma

The clinical characteristics and prognosis of the 127 MCL patients included in this study support what has previously been published in smaller patient series [9, 20, 21, 100, 113]. Typically, the MCL occurs in elderly patients at an advanced stage. In addition to generalised lymphadenopathy, involvement of extranodal sites, especially the bone marrow, was frequently seen at the time of diagnosis. However, the performance status of the patients was usually good and only one third of the patients presented with B symptoms. In contrast to the reported great preponderance of males (up to 94%) in most other studies [7, 9, 11, 100, 190, 191], only 64% were males in the present study.

Gastrointestinal involvement at presentation occurs in 15 to 30% of the patients as assessed endoscopically [9, 11, 12, 14, 20, 114]. In this study gastrointestinal lymphoma was detected in 20% of the patients at the time of diagnosis. However, gastrointestinal MCL seems to be highly underestimated if endoscopy is performed only as directed by symptoms: recently gastrointestinal tract was shown to be affected in no less than 88% of 32 consecutive patients, who underwent systematic endoscopic examinations regardless of whether gastrointestinal symptoms were present or not [192]. The authors suggested that upper and lower gastrointestinal tract endoscopies should be considered as a staging procedure at presentation also in patients who have no gastrointestinal symptoms.

The long-term prognosis of MCL is poor and this was confirmed in the present study: the median overall survival was only 34 months. There was no plateau in the survival curve, and only one fourth of the patients were alive five years after diagnosis. Despite this poor overall outcome, many clinical and histopathological factors were found to have clinically significant prognostic value.

Prognostic significance of tumour characteristics and clinical factors

The present study showed that the three histological subtypes of MCL have prognostic significance (study IV). The subtypes are the mantle zone/nodular subtype of the common variant (19% of all cases), the diffuse subtype of the common variant (64%), and the blastoid variant (17%). The mantle zone/nodular subtype was associated with the most favourable outcome (median survival 70 months) followed by the diffuse subtype of the common variant (median survival 35 months), whereas outcome was the poorest in patients who presented with the blastoid variant (median survival 11 months).

As summarised in Table 12, several other studies, in addition to the present one, have shown a highly aggressive clinical course of the blastoid variant of MCL [7, 9-13]: median survival times of 1-2 years have been reported in all except one study (55 months) [152]. In that study, the CR-rate was higher among the patients with the blastoid variant than among the patients with the other histological types, and this may partly explain why the patients had a survival that was longer than is usually the case for patients with the blastoid variant. In the present study there was no significant difference regarding the intensity of the primary treatment or the CR-rate among the patients with the different histological subtypes. Rather, the patients diagnosed with the blastoid variant presented more often with other unfavourable clinical prognostic factors, e.g., older age, extranodal involvement, and B symptoms than did the patients presenting with the other histological subtypes.

More conflicting results have been reported concerning the clinical significance of the various growth patterns of MCL. As shown in Table 12, the architectural pattern of MCL has had prognostic significance in some of studies but not all. The mantle zone pattern has been associated with long survival times in some older studies [193, 194]. However, the number of patients included in these studies has been relatively small, and other types of B-cell lymphoma, such as MALT lymphomas, may have been included, since immunohistochemical confirmation of the diagnosis has not always been possible or available. More recently, there has been only one report of an outcome benefit of patients presenting with the mantle zone subtype [114]. On the other hand, Fisher *et al.* [7] have reported poorer outcome in 10 patients who had the diffuse growth pattern of MCL as compared to 14 patients who had the mantle zone or nodular growth pattern, and a similar trend was also seen in the study of Argatoff *et al*. [10] (Table 12). These reports agree with the present study.

It is difficult to compare the clinical significance of the different histological subtypes between studies, because there are no uniform standardised diagnostic criteria for the different subtypes. The criteria for classification of the growth patterns are not clearly reported in many studies, and the rather common vaguely nodular pattern may have been classified as nodular in some and as diffuse in other reports. In the present study, the growth pattern was classified as mantle zone or nodular only when such a definite pattern was seen in over 50% of the tissue examined. The MCL of only two of our patients had the mantle zone pattern, and the data were analysed together with those who had the nodular pattern, as has been done in some other studies, as well [7, 10, 152]. Also, different methods of case selection have obviously used in the different studies, as the knowledge of the morphological heterogeneity of MCL has gradually increased over the last decade. These problems clearly reflect the high variance in the proportion of the different histological subtypes and their controversial clinical significance reported in the various studies on MCL (see Table 12).

The present study (study IV) showed also that a high mitotic index and a high

Ki-67 expression were strongly associated with the blastoid variant morphology and a poor outcome, which is in accordance with earlier studies [10, 11, 18, 93, 113, 178]. The present work also showed that the cell proliferation rate varies strongly from one lymphoma to another, and that some common variant MCLs can also have a high rate of cell proliferation. An interesting observation was that a high Ki-67 expression was associated with a short survival also in a subset of patients with the common variant.

Overexpression of p53 protein correlated with the blastoid variant and a poor outcome (study IV). However, unlike lymphomas with a high mitotic index or high Ki-67 expression, lymphomas with overexpression of p53 responded poorly to chemotherapy, which probably explain the adverse prognostic influence of p53 expression. Interestingly, loss of p53 function has been found to be strongly associated with clinically resistant and aggressive tumours in general [66].

In addition to the tumour characteristics, this study identified many clinical factors as indicators of a poor prognosis. Most important of these factors were age over 60 years, advanced stage, presence of B symptoms, splenomegaly, high IPI, bone marrow infiltration, leukaemic disease, low haemoglobin level, leukocytosis, lymphocytosis, and a high level of LDH in the serum at diagnosis. Most of these factors have been prognostic indicators in previous studies (see also Table 3), although bone marrow involvement and B symptoms have not previously been agreed as having prognostic significance in MCL [3, 9-11, 14, 21]. Nevertheless, the independent prognostic significance of these factors has been poorly characterised, especially in relation to the histopathological features. Multivariate analyses encompassing the tumour characteristics according to the architectural pattern, the cytological variant, and cell proliferation activity have not been performed previously.

Independent prognostic factors. The IPI is one of the most popular prognostic indicators of NHLs. It was originally proposed for predicting outcome in large cell diffuse and immunoblastic lymphomas, but it has been adopted as a prognostic tool also in other types of NHLs [179, 180]. The IPI may have prognostic value also in MCL, although rather conflicting results have been reported [11, 14, 21, 113, 115, 152]. The present findings suggest that the IPI components selected on the basis of survival analyses in other types of NHLs may not be optimal for MCL (studies I, IV). Firstly, although the IPI had a strong prognostic correlation with patients outcome, the IPI could not discriminate survival between the low-intermediate and high-intermediate risk groups. Secondly, although all IPI components, except for the number of extranodal disease sites had prognostic significance on outcome by univariate analyses, it was only age, stage, and LDH that contributed significantly to survival when all IPI components were analysed together in Cox's proportional hazard regression model. Thirdly, the histological subtype and Ki-67 expression were found to improve the prognostic model when analysed together with the IPI components in Cox's multivariate analysis, but two of the IPI components, the performance status and the number of extranodal disease sites had no

Table 12. *The incidence and prognostic significance of the cytological variant and the architectural pattern of lymphoma in patients with mantle cell lymphoma reported in the recent literature.*

Variable	Present study	Fisher *et al.* [7] (1995)	Norton *et al.* [9] (1995)	Pittaluga *et al.* [100, 118] (1996)	Argatoff *et al.* [10] (1997)	Majlis *et al.* [114] (1997)	Samaha *et al.* [14] (1998)	Bosch *et al.* [11] (1998)	Yatabe *et al.* [12] (2000)	Weisenburger *et al.* [152] (2000)	Bernard *et al.* [13] (2001)
All patients											
N of patients	127	36	66	55	80	46	121	59	128	68	187
Median follow-up (months)	87	NR[a]	NR	NR	39	22	NR	29	NR	18[b]	24[b]
Median OS (months)	34	NR	36	32	43	NR	37	49	30%[c]	38	NR
Incidence (%)											
MZ	2%	-	9%[e]	13%[e]	6%[e]	26%[e]	0	0	17%[e]	22%	-
Nodular	17%	39%[d]	30%[e]	-	16%[e]	13%[e]	34%[e]	5%	38%[e]	22%	-
Diffuse	64%	28%	61%[e]	87%[e]	78%[e]	61%[e]	66%[e]	85%	45%[e]	24%	-
Blastoid variant	17%	33%	15%[e]	2%[e]	6%[e]	4%[e]	8%[e]	10%	16%[e]	26%	21%
Median OS (months)											
MZ, common	-	-[i]	NR[g]	98	-	100%[h]	-	-	-	-[i]	-
Nodular, common	70[d]	60[d, f]	NR[g]	-	47[d]	50%[h]	NR[g]	-	-	50[d]	-
Diffuse, common	35	36[f]	NR[g]	41	39	50%[h]	NR[g]	-	-	15	-
Blastoid	11	16[f]	-	-	15	-	NR[g]	-	-	55	-
P value	< 0.001	0.05	NS	NS	NS	NR	NS	-	-	0.001	-
Median OS (months)											
Common variant (all)	35		42		45		NR[g]	50	48		53
Blastoid variant	11		24[j]		15		NR[g]	18	14		15
P value	< 0.001		0.02		0.001		NS	0.007	< 0.001		< 0.001
Blastoid transformation N / Evaluated (%)	18 / 52 (35%)	-	11 / 50 (22%)	3 / 16 (19%)	8 / 37 (22%)	-	24 / 82 (29%)	-	-	-	-

OS: overall survival; MZ: mantle zone pattern; NR: not reported; NS: not significant

[a] All patients have died

[b] Median follow-up reported for the surviving patients

[c] Five-year survival

[d] MZ and nodular cases included

[e] The proportion of different growth patterns have been reported including the blastoid variant of MCLs

[f] Estimated on the basis of the survival curve

[g] Reported as no difference in outcome

[h] Three-year survival

[i] No difference in outcome between patients with the MZ and nodular growth pattern of MCL

[j] Median OS reported for cases showing the blastoid variant of MCL at any time during the course of the disease

independent prognostic value. It is clear that the current study is too small to establish any reliable prognostic model in MCL, but the findings strongly suggest that a better prognostic tool might be obtained by including a factor related to the cell proliferation rate (as Ki-67 expression) and, possibly, the histological subtype as components in the index (together with age, stage, and serum LDH). A multicentre collaborative study is needed to verify the general validity of the present findings, and to identify the optimal set of factors for a prognostic index specifically designed for MCL.

Chromosomal features

The characteristic genetic abnormality in MCL, t(11;14) or the *BCL1* rearrangement was found in 38% of the studied 24 patients (study III). We studied the translocation breakpoints only for the MTC region, which covers 30-50% of the known breakpoints. This explains the relatively low frequency of detected translocations in this study as compared to recent studies, which have shown that this translocation is present in nearly all MCLs [37, 38]. These studies have used a variety of FISH techniques with a proper set of probes.

As t(11;14) leads to overexpression of cyclin D1, which regulates the cell cycle in the early S-phase, cell proliferation markers might be powerful prognostic factors in MCL. In line with this hypothesis, we have shown that a high cell proliferation rate is an independent predictor of a poor prognosis of patients with MCL (study IV). However, the cell proliferation rate is usually relatively low in MCL, and no correlation between the level of cyclin D1 overexpression and cell proliferation rate has been detected [32, 56, 60]. Thus, although cyclin D1 certainly has a crucial role in the pathogenesis of MCL, it alone seems not explain the poor prognosis of these patients.

Conventional karyotype analyses have shown that MCLs have very complex and, in many cases, chaotic chromosome abnormalities [90]. Indeed, individual changes may be very difficult to detect by G-banding analysis. CGH provides a method to study changes in the DNA copy number of the whole genome based on simultaneous hybridisation of differentially labelled tumour and normal DNAs on normal metaphase chromosomes. In our series of the 22 common variant and 12 blastoid variant of MCL studied by CGH (study III), complex but highly concentrated DNA copy number changes were seen. DNA copy number changes were found in all cases, and eight regions were involved in more than 25% of the cases. The most common changes were gains at 3q, 8q, and 15q, and losses at 13q, 1p, 6q, 9p, and 11q. Most of the individual alterations have been observed also in other NHLs, but the profile and frequency of the imbalances seem to be relatively characteristic for MCL [76, 77, 195-201]. Furthermore, the profile of the chromosomal abnormalities reported in later MCL series studied by CGH are well in accordance with the present findings [72, 73, 75].

In previous studies the blastoid variant of MCL has been associated with more cytogenetic or molecular abnormalities, such as tetraploid chromosome clones, mutations or deletions of p53, and inactivation of negative cell cycle regulatory proteins than the common variant of MCL [16, 68, 69, 178]. In agreement with this, the present study showed that the median number of DNA copy number changes tended to be higher in the blastoid type than in the typical MCL cases. All five lymphomas showing high-level amplifications in the present study were of the blastoid type. Later, according to the observations of a larger number of patients, the number of chromosome imbalances and DNA amplifications were reported to be significantly more frequent in the blastoid than in the typical tumours, and gains of 3q, 7p, and 12q, and losses of 17p were seen more often in the blastoid variant than in the common variant [72]. In that study also gains of 3q and 12q, and losses of 9p were associated with a shorter survival.

Two of the frequent aberrations detected by CGH analysis, gain of 3q and deletion of 11q, were of specific interest (study III). Trisomy 3 is one of the most common numerical aberrations in NHL and is typical of the diffuse large B-cell lymphoma [202, 203]. Trisomy 3 has been found previously only in a few MCL tumours by conventional cytogentic analysis [204]. In the present series, gain of 3q was the most common aberration and it was detected in 52% of the patients with the minimal common region located at 3q26.1-27. Although the Spanish group found the gain of 3q to be more frequent in the blastoid than in the common variant MCL [72], no less than 70% of the patients with the common MCL variant showed this aberration in a recently published series [75]. Accordingly, this area may harbour an oncogene or oncogenes that are important for initial lymphomagenesis or progression in MCL. Zinc finger protein *BCL6/LAZ3*, which is known to be activated by translocation in some B-cell lymphomas, has been mapped to 3q26-27 [205, 206], but amplifications or rearrangements of the *BCL6* gene have been found to be virtually absent in MCLs [72, 207].

Deletion of the long arm of chromosome 11 is one of the most common chromosomal aberrations observed in lymphoid neoplasms [208-210]. In the present study, losses of 11q14-24 were found in 30% of the cases with the minimal common region mapped to 11q22. LOH analysis supported this result, as all the markers exhibiting LOH were mapped to 11q22-23. In further studies using interphase FISH, a deletion at this region has been found in up to 49% (20/41) of MCLs [78]. Consistent with these results, deletion of the same common minimal region in 11q has been reported frequently also in CLL [209, 211] and diffuse large cell lymphoma [212], suggesting that this area is a potential candidate region for a tumour suppressor gene involved in MCL as well as in CLL and diffuse large cell lymphoma. The region 11q22-23 is a very gene-rich area and contains several known or putative malignancy associated genes. Recently, the *PPP2R1B* gene was shown not to be the likely target of 11q22-23 deletion in MCL or CLL [213], but one of the suggested tumour suppressor genes in this region is the *ATM* gene, which is known to code for a protein that is critically involved in the cellular response to DNA damage. Ataxia-telangiectasia patients with homozygous *ATM*

mutations have an increased risk of leukaemias and lymphomas [214]. Furthermore, frequent inactivating mutations of the *ATM* gene have been reported in patients with T-PLL [80-82] and CLL [83, 84, 215]. More recently, *ATM* mutations have been identified also in MCLs associated mainly with 11q22-23 deletions, and with a same frequency both in the common and the blastoid variant MCL [86, 87, 216, 217]. Altogether these results suggest that *ATM* gene may be involved in early steps of tumour development in MCL and other malignancies.

In accordance with previously reported cytogenetic findings [218], we also found more changes in samples taken from the relapsed lymphomas than in those taken from untreated lymphomas of the same patients. To our knowledge, the DNA copy number changes studied by CGH have not been compared between the primary and recurrence MCL tumours elsewhere. Although the number of patients with sequential samples was small in our study, interestingly, some changes including the deletion at 11q and the gain on 3q, were always seen both in the primary and recurring lymphomas. Taken together with the other results this indicates that the deletion of 11q and gain of 3q may be early changes in MCL.

Clinical significance of blastoid transformation

In addition to patients who present with the blastoid variant of MCL at diagnosis, there is a remarkably large proportion of patients who have cytological transformation of the common variant to the blastoid variant during the course of the disease. In accordance with earlier findings [9, 10, 14, 100] (see Table 12), such a change was found in 18 (35%) of the 52 patients who had sequential biopsies taken at the time of disease progression (study V). The minimum estimated risk of blastoid transformation was 24% at 3 years and 42% at 5 years. In another study blastoid transformation has been reported in 14 (70%) of the 20 autopsy-verified cases [9].

In line with the poor outcome of patients presenting with the blastoid variant of MCL, overall survival was also significantly shorter among the patients who had blastoid transformation during the course of the disease (median survival 31 months) compared with patients who did not have this transformation at rebiopsy (median survival 60 months) (study V). Blastoid transformation at diagnosis or at any time during the course of the disease has been shown to be associated with a highly aggressive clinical course in one of the previous studies [9]. In two other studies where survival was assessed, blastoid transformation was not found to have a significant effect on outcome [10, 14], although the blastoid morphology at diagnosis did predict an unfavourable prognosis in one of these studies [10]. The authors suggested that a lead-time bias may explain the similar outcome following rebiopsy between patients with and without transformation, since rebiopsy was taken remarkably later after the initial diagnosis of patients with transformation than of patients without. In contrast, the time interval between the

sequential biopsies was roughly similar in patients with (median 32 months) and without (median 26 months) detected transformation in the present study.

Interestingly, an increased risk of blastoid transformation during the course of the disease was seen in patients with blood leukocytosis, an elevated serum LDH level, and a high cell proliferative activity of lymphoma at the time of the diagnosis. Blastoid transformation occurred also earlier after diagnosis in patients with leukocytosis and elevated LDH. To our knowledge, factors predictive of blastoid transformation have not been analysed previously.

As described in the previous chapter, more cytogenetic or molecular abnormalities have been detected in the blastoid than in the common variant MCL. However, the underlying molecular pathogenesis related to the development of blastoid transformation de novo or later during the course of the disease is still unknown. Several findings support a hypothesis that aberrations of the *p53* gene might be involved in such progression of MCL. Firstly, mutations of *p53* and overexpression of p53 protein occur more often in lymphomas with the blastoid than the common variant [15-17]. Also in the present study blastoid MCLs at presentation tended to show overexpression of p53 protein more frequently than the common MCLs (40% vs. 15%) (study IV). Secondly, the occurrence of *p53* mutations has been suggested as one mechanism in the transformation of CLL or follicular lymphoma into the diffuse large B-cell lymphoma [219-221]. In line with this concept, we observed that two of the three patients who had the common variant of MCL with p53 overexpression at diagnosis developed blastoid transformation during the course of the disease (study V). Similarly, Greinen *et al.* [16] reported progression from the common to the blastoid variant in two of the four MCL patients showing the *p53* mutation. However, further molecular studies based on serial biopsies are needed to confirm the role of p53 and to establish other putative mechanisms that may be involved with blastoid transformation.

Importantly, the present findings suggest that development of CNS lymphoma in MCL is relatively frequent and strongly related to the blastoid morphology. Although CNS involvement has been considered to be very rare in small cell lymphomas [129-132], the disease relapsed with CNS involvement in seven (6%) of the total of 127 patients during follow-up. Five of these patients had the blastoid variant of MCL at the time CNS lymphoma was diagnosed. The prognosis after CNS relapse was very poor regardless of intrathecal chemotherapy. The findings by Montserrat *et al.* [11, 135] are in accordance with those of ours: there were seven (12%) CNS relapses in 59 patients with MCL.

Treatment results

Conventional therapy. So far, there is no generally approved standard therapy for MCL. This explains why the patients in our series, as well as in other retrospective

series, have received rather heterogeneous treatments. In the present study, most of the patients (76%) received anthracycline-containing regimens (or equivalent) as first-line treatment, and 20% were given chlorambusil with or without prednisone, or COP/CVP. Six of the patients did not receive active treatment for their lymphoma, and the remaining patients were treated by local radiotherapy only, or local disease was operated. Although almost one half of the actively treated patients achieved a CR, relapses occurred usually soon: the median duration of remission was only about 1.5 years. None of the conventional chemotherapeutic regimens was curative. First-line treatment with anthracycline-containing chemotherapy showed neither significant benefit on CR-rate, TTF nor survival as compared with non-anthracycline containing regimens.

As stated in the review section (pages 24 - 26), and in line with our findings the results of treatment with conventional therapy have been poor in MCL, and there was no clear improvement in the results with regimens containing anthracyclines as compared with regimes without anthracyclines. However, most of the published studies on the management of MCL have included relatively small number of patients, and only two small prospective trials in MCL have been carried out [138, 139]. In one retrospective study including 65 MCL patients, anthracycline-containing regimens showed a survival benefit in patients of the low- or low-intermediate risk IPI categories [21]. None of the other series have been able to confirm a survival benefit of anthracylines in MCL [9, 11, 14, 20, 138, 139]. Interestingly, the Hyper-CVAD regimen has yielded considerably better treatment results than traditional chemotherapy regimens; here historical controls were used [149, 150]. However, it can be concluded that none of the current conventional chemotherapies are curative in MCL, and more accurate and efficient therapies are needed. During recent years, promising results have been published in some preliminary reports of small non-randomised MCL series using high-dose therapy and autologous or allogeneic stem cell transplantation [149, 155-159, 162, 167], but the role of stem cell transplantations in treating MCL is not yet clear.

Autologous stem cell transplantation. In the present study, the results of high-dose therapy supported with ASCT were retrospectively analysed in a series of 48 consecutive patients treated in five Finnish transplant centres in 1995 to 2000 (study VI). Altogether, 35 out of 48 scheduled patients underwent ASCT, 24 as a part of first-line treatment and 11 patients later as a part of subsequent therapy. The overall response rate was 94% in these patients, and the expected four-year PFS was 50% and overall survival 69% following ASCT, respectively. There was, regrettably, no plateau in the EFS curve, and late relapses occurred even three years after the ASCT.

In line with our findings, high CR-rates after ASCT have been reported in most previous studies [149, 155-160, 162, 222]. Most of these studies have concluded that ASCT is an efficient treatment of patients with MCL [149, 155-159, 162], although the survival probabilities after ASCT varies widely between various studies. Comparison

between these retrospective studies is difficult because of variable patient selection. The number of included patients has been relatively small (only a few series include more than twenty patients), and the median follow-up time after transplantation has exceeded three years in none of the series. Most interestingly, the preliminary results of the first prospective randomised trial comparing ASCT and α-interferon maintenance as a part of first-line therapy in chemosensitive patients showed a significant prolongation in EFS in the group of patients treated with ASCT [163]. However, the follow-up time is still too short to show a significant difference of overall survival between these patient groups. In accordance with our findings, the prospective study also showed no plateau in the EFS curve after ASCT, at least not thus far. Although the prognosis in MCL may be improved with high-dose therapy supported by ASCT, late relapses do occur, and it is not yet known in what proportion of patients continuous remissions may take place.

Graft contamination has been considered to be a considerable problem in ASCT to treat lymphomas with bone marrow involvement [164, 223]. Another likely cause of relapses after ASCT is the persistence of residual disease despite of the use of high-dose therapy. It is not known if selection of the autograft could provide a better outcome after ASCT in MCL. Only two out of 11 patients treated with a CD34+ -selected graft in the present series has progressed, but no significant difference in outcome was seen as compared with the patients who had a non-selected graft (study VI). However, effective immunological purging of the graft below the level of PCR detection has been shown to be more difficult in MCL than in other lymphomas [165, 224]. Interestingly, preliminary reports claim that there was a high number of molecular responses in blood and bone marrow of MCL patients who got in-vivo purging using the anti-CD20 antibody, rituximab, in combination with the ASCT [225-228]. Whether rituximab or other immunotherapies in combination with high-dose therapy can improve the long-term outcome of MCL patients remains to be seen.

The ideal timing of ASCT to treat MCL and the prognostic factors affecting the outcome following ASCT are still poorly known. Although the results of some studies have supported early high-dose treatment [149, 160], our study indicated that the outcome of the patients transplanted after relapse was similar to that of the patients with ASCT performed during the first- or second-line treatment (study VI). However, the patients who were transplanted after relapse represent a more selected patient group with a response to therapy for the recurrent disease and a successful mobilisation of stem cells. A blastic morphology and three or more chemotherapy regimens prior to transplantation have been previously reported to predict a shorter EFS after ASCT [229]. In the present study, splenomegaly, gastrointestinal tract or bone marrow involvement, and a high CRP level at the time of diagnosis of MCL were associated with a poor outcome after ASCT. Also patients over 60 at the time of transplantation had a poorer outcome than younger patients.

A satisfactory number of progenitor cells may be sometimes difficult to mobilise [230, 231]. Of notice, in the present study, mobilisation of PBSCs failed in 29% (14/48) of the patients who were originally intended for treatment with ASCT (study VI). Most of the patients received cyclophosphamide or CHOP as mobilisation regimens together with G-CSF. There was no significant difference in mobilisation failures between cyclophosphamide and CHOP, or the other regimens. Neither did the dose of G-CSF (5 vs. 10 μg s.c) have any impact on mobilisation success. Instead, female sex, blastoid cytology, and a low haemoglobin level shortly before the start of the mobilisation therapy were associated with the mobilisation failure. Most patients who had achieved only a few chemotherapy cycles before mobilisation therapy had other prognostic factors for an unsuccessful mobilisation, which may explain why the number of prior chemotherapy cycles was not associated with the mobilisation failure in the present study. However, the number of prior chemotherapy cycles affected adversely the number of harvested CD34+ -cells, which is in accordance with previously reported results on PBSC harvesting in lymphomas and other malignancies [232-234]. No predictive factors for mobilisation failure in MCL seem to have been previously reported, but in line with our finding, Khouri *et al.* [149] have reported that nine (31%) out of 29 MCL patients considered for ASCT failed to have an adequate number of PBSCs following G-CSF mobilisation. Importantly, successful mobilisation of PBSCs in MCL seems to be complicated in a remarkable proportion of the patients. As evidence of the importance of intensive treatment supported by ASCT in the treatment of MCL is increasing, more efficient mobilisation therapies are needed to make ASCT available for all potential patients who otherwise tolerate intensive treatment.

SUMMARY AND CONCLUSIONS

The main conclusions of the present study are:

1. Confirming the findings of previously published smaller series, MCL presented typically in elderly patients with an advanced stage disease and often with extranodal involvement. Although almost half of the patients achieved complete remission with conventional chemotherapy, the duration of the ensuing remission was usually short and the general outcome was poor: the median overall survival was approximately 3 years.

2. The blastoid variant of MCL is associated with a high cell proliferation rate and an aggressive clinical course according to previous studies. The present study showed that also other tumour characteristics at presentation have considerable prognostic significance. Three histological subtypes of MCL were characterised: 1) the mantle zone/nodular subtype of the common variant was associated with the most favourable outcome followed by 2) the diffuse subtype of the common variant, whereas 3) the blastoid subtype was associated with the most aggressive clinical course and a median survival of less than one year. Furthermore, Ki-67 expression was an important overall prognostic factor, and also separately among the patients with non-blastoid MCL.

3. The impact of the histopathological features of MCL on patient outcome in conjunction with clinical factors has not been previously established. Of the clinical factors, an age over 60, advanced disease stage, and an elevated serum LDH level at diagnosis were independent prognostic factors of a poor outcome. Importantly, the prognostic model improved when Ki-67 expression or the histological subtype was analysed together with the five components of the IPI. This study showed that IPI may not be an optimal prognostic tool for MCL, and a better prognostic model might be developed by including Ki-67 expression and, possibly, the histological subtype in the index.

4. The risk of CNS involvement was higher in MCL than is generally considered in small cell lymphomas. Importantly, this study showed that CNS relapse was strongly associated with the blastoid morphology.

5. Transformation from the common to the blastoid variant during the course of the MCL is know to occur, but the clinical significance of blastoid transformation has not been established. In this study the minimum estimated risk of blastoid transformation was over 40% at 5 years, and blastoid transformation contributed significantly to a poor prognosis in MCL. The risk of transformation was higher in

patients with elevated serum LDH level, leukocytosis, and a high cell proliferation of lymphoma at diagnosis than in patients who did not exhibit these characteristics.

6. Study III was the first one in the literature to examine the DNA copy number changes by CGH in MCL. The study showed a characteristic profile of chromosomal changes: the most common gains were located at 3q, 8q, and 15q, and the most common losses at 1p, 6q, 9p, 11q, and 13q. In concordance with a more aggressive clinical course, the number of changes tended to be higher in the blastoid than in the common variant, and high-level amplifications occurred only in the blastoid cases. More changes were also seen in relapsed lymphomas than in untreated lymphomas of the same patients. Most importantly, the finding of a small, commonly deleted region in 11q22 has resulted in further investigations to identify a tumour suppressor gene, which could be involved in the pathogenesis of MCL and other malignancies. Also, the gain of 3q, which was seen in more than half of the patients, indicates that this area may contain an oncogene or oncogenes important for tumour initiation or progression in MCL.

7. Patients who were treated with the high-dose therapy supported by ASCT had higher response rate and longer survival times than in conventionally treated MCL patients in general. Together with previous data, this confirms the ASCT to be en effective treatment in MCL, although possible survival benefit remains to be studied in prospective trials. However, this study showed no plateau in the event-free survival curve after ASCT, but relapses were seen even after three years. This study can not answer the question of whether continuous remissions can be reached by ASCT in a proportion of the patients or not.

This study has shown that several tumour characteristics and clinical factors have considerable prognostic significance in MCL, and that there is a need for a new prognostic model designed for MCL to be identified in a multicentre collaborative study. It is likely that our improving understanding of the genetic changes related to MCL will aid the evaluation of the patients' prognosis. Ongoing studies on gene expression profiling using novel micorarray technologies are likely to widen the knowledge of the pathogenesis of MCL, as well as to identify the genes related to specific disease characteristics, like blastoid transformation. Hopefully these studies will also widen our understanding of the mechanisms related to treatment resistance and the generally poor prognosis of patients with MCL. Ultimately this approach may provide a platform for the development of novel, more accurate treatment strategies.

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